Journal

OF THE

ELISHA MITCHELL Scientific Society

VOL. 70, NO. 2 DECEMBER 1954

Contents

PROCEEDINGS OF THE FIFTY-FIRST ANNUAL MEETING OF THE NORTH CAROLINA ACADEMY OF SCIENCE 105
PROCEEDINGS OF THE ELISHA MITCHELL SCIENTIFIC SOCIETY 134
THE LIFE HISTORY OF THE NORTHERN GREENSIDE DARTER, ETHEOSTOMA BLENNIOIDES BLENNIOIDES RAFINESQUE ..... *William E. Fahy* 139
STRUCTURE AND RELATIONSHIPS OF THE ANGLERFISH, LOPHIUS AMERICANUS *Theodore H. Eaton, Jr., Charles A. Edwards, Margaret Ann McIntosh, and Joseph Perry Rowland* 205
AN EXPERIMENTAL STUDY OF THE "BARRED" PIGMENT PATTERN IN MEXICAN AXOLOTL LARVAE ..... *H. E. Lehman* 218
A SECOND RECORD OF MYOTIS LEIBI IN NORTH CAROLINA ..... *Albert Schwartz* 222
VITAMIN A AND TUMOR GROWTH IN GONADECTOMIZED, METHYLCHOLANTHRENE-INJECTED MICE ..... *B. Lionel Truscott* 222
COMMENTS ON THE TEACHING OF TRIGONOMETRY ..... *T. F. Hickerson* 228
THE UTILIZATION OF CARBON AND NITROGEN COMPOUNDS BY PHYTOPHTHORA PARASITICA DASTUR VAR. NICOTIANAE (BREDA DE HAAN) TUCKER *Wirt H. Wills* 231
SPORANGIUM FORMATION BY PHYTOPHTHORA PARASITICA DASTUR VAR. NICOTIANAE (BREDA DE HAAN) TUCKER ..... *Wirt H. Wills* 235
TOXICITY AS A FACTOR IN TOBACCO BLACK SHANK *Frederick T. Wolf and Frederick A. Wolf* 244
THE TRANSPIRATION OF BLACK SHANK-INFECTED TOBACCO *Robert J. Schramm, Jr. and Frederick T. Wolf* 255

Continued on outside back cover

Published for the Society by

THE UNIVERSITY OF NORTH CAROLINA PRESS *at* CHAPEL HILL

The Elisha Mitchell Scientific Society

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JOURNAL OF THE ELISHA MITCHELL SCIENTIFIC SOCIETY—Semi-annual. Price $3.50 per year; single numbers, $1.75. Direct all correspondence to the Corresponding Secretary, at the University of North Carolina, Chapel Hill, N. C.

In addition to original papers on scientific subjects, this Journal publishes the Proceedings of the Elisha Mitchell Scientific Society, and the Proceedings of the North Carolina Academy of Science, including some abstracts of papers on scientific subjects published elsewhere.

PUBLISHED FOR THE SOCIETY
BY THE
UNIVERSITY OF NORTH CAROLINA PRESS

Made in the United States of America

JOURNAL
OF THE
Elisha Mitchell Scientific Society

Volume 70 December 1954 No. 2

PROCEEDINGS OF THE FIFTY-FIRST ANNUAL MEETING OF THE NORTH CAROLINA ACADEMY OF SCIENCE

The fifty-first annual meeting of the North Carolina Academy of Science was held at East Carolina College, Greenville, on May 7 and 8, 1954.

The Academy met in its first session at 10:00 a.m. on May 7 with President Donald B. Anderson presiding. A symposium on Growth and Development was presented at this session.

The business meeting of the Academy was held at 4:30 p.m. at which the following business was transacted:

The minutes of the 1953 meeting were approved as published in the Journal of the Elisha Mitchell Scientific Society **69:** 63–99.

Report of the Executive Committee

The Executive Committee met at East Carolina College on May 6 at 7:30 p.m. At this meeting the following matters were considered:

1. The committee noted with pleasure the interest shown this year in the first program of the Collegiate Academy and voted to underwrite the expense of promoting this new section for another year. They also voted that the Academy offer the same prizes, $25 and $15, as this year unless a donor can be found to assume this responsibility.
2. It voted to accept the offer from the A.A.A.S. to offer honorary annual memberships in that organization to two members of the Collegiate Academy and to two high school students who present exhibits at the annual meeting.
3. The Secretary reported the following facts about membership:

1. Loss by non-payment of dues (1953)	36
2. Loss by resignation (1954)	6
3. Gain—new members and reinstatements	73
4. Total membership for 1953	501

4. It voted to dispense with the reading of names of new and reinstated members at the annual business meeting since these are published in the Proceedings.
5. The following were elected to membership in the Academy or reinstated to active membership:

Bank, Walter, U. S. Bureau of Mines, Raleigh
Barnett, Mrs. Annie R., Andrews
Barber, Yates M., Jr., Wildlife Resources Commission, Raleigh

Bates, Dr. William W., Liggett and Myers, Durham
Bethune, Paul P., Charlotte
Blackburn, Mrs. Rena P., Tarboro
Borg, Dr. Alfred F., N. C. State College, Raleigh
Brock, Mrs. Barbara Y., New Bern
Brown, Philip Monroe, U. S. Geological Survey, Raleigh
Bryan, Mrs. Virginia S., Duke University, Durham
Butler, Howard
Childs, Charles Benson, University of N. C., Chapel Hill
Collier, Jack R., University of N. C., Chapel Hill
Constable, Dr. Ernest W., N. C. Dept. of Agriculture, Raleigh
Cook, Miss Doris Jean, Kannapolis
Davis, Graham J., University of N. C., Chapel Hill
Ennett, A. D., Wildlife Resources Commission, Raleigh
Fishel, Miss Katherine H., Lexington
Fitzgerald, Miss Hannah T., Kinston
Forrest, Mrs. J. Everette, Hillsboro
Fowler, Dr. Ira, University of N. C., Chapel Hill
Freeman, Oliver M., Tryon
Hamrick, Asa Harrill, Kenansville
Helms, Mrs. Zelian, Raleigh
Heneage, Peter, Duke University, Durham
Hiatt, Dr. E. P., University of N. C., Chapel Hill
Hill, Dr. Charles H., N. C. State College, Raleigh
Howard, Clarence E., N. C. State College, Raleigh
Hoyle, V. A., University of N. C., Chapel Hill
Jenzano, Anthony F., Morehead Planetarium, Chapel Hill
Jones, Franklin R., Henderson
Kersey, Robert L., Jr., Liggett and Myers, Durham
LaChance, Leo E., N. C. State College, Raleigh
LeGrand, John R., Atlantic Coast Line R. R. Co., Wilmington
Ljung, Dr. Harvey A., Guilford College, Guilford
Martin, Miss Elizabeth G., Warren Wilson College, Swannanoa
Melvin, Lionel, Pleasant Garden
Moffitt, Dr. James W., High Point College, High Point
Monroe, Dr. Robert J., N. C. State College, Raleigh
Moore, Henry, Liggett and Myers, Durham
Moorman, Dr. Albert E., Catawba College, Salisbury
Morgan, Voigt F., Spray
Mumford, Dr. C. G., N. C. State College, Raleigh
Nixon, Edward C., N. C. State College, Raleigh
Owens, Mrs. Eleanor B., Goldsboro
Owens, Daniel K., N. C. State College, Raleigh
Owens, Laura E., Charlotte Nature Museum, Charlotte
Perkins, Kenneth W., High Point College, High Point
Pitts, Charles R., N. C. State College, Raleigh
Polk, Clyde F., Jr., Louisburg
Powers, Maurice C., University of N. C., Chapel Hill
Price, Thomas J., U. S. Shellfish Laboratory, Beaufort
Pruette, Churchill R., Louisburg College, Louisburg
Reeves, Walter P., Jr., Warren Wilson College, Swannanoa
Reynolds, Dr. Thomas D., Duke University, Durham
Roberson, John L., Parmele
Rossy-Valderrama, Miss Carmen R., Duke University, Durham
Schipf, Robert G., U. S. Geological Survey, Raleigh

Scott, Harry E., N. C. State College, Raleigh
Shaw, Aubrey M., Maysville
Smart, Dr. William W. G., N. C. State College, Raleigh
Sprague, Dr. Victor, Hiawassee, Georgia
Steed, Frank W., Jr., Mt. Airy
Tebo, Lee B., Wildlife Resources Commission, Raleigh
Waters, Gerald, Seven Springs
Weaver, Dr. Richard L., University of Michigan, Ann Arbor, Michigan
Wells, Mrs. Nellie S., Trenton
Whatley, J. W., Jr., Durham
Whitehead, Laurence C., U. S. Fish and Wildlife Service, Raleigh
Wiggins, Dr. John C., Jr., Bowman Gray School of Medicine, Winston-Salem
Wilfong, Dr. Robert E., E. I. du Pont de Nemours & Co., Kinston
Winton, Dr. Lowell S., N. C. State College, Raleigh
Wise, Dr. George H., N. C. State College, Raleigh

6. The committee recommended Life Membership in the Academy for Dr. Otto Stuhlman, Emeritus Professor of Physics, University of North Carolina, and past president of the Academy, and for Dr. R. L. Fritz, Emeritus Professor of Mathematics and former President of Lenoir-Rhyne College.
7. It voted to increase the annual dues for Academy members to $3.00.
8. It voted to approve the changes in the constitution of the Academy recommended by a special committee appointed by President Anderson. The changes in the constitution are as follows:

Art. I. Section 2—from "to promote study and scientific research" to "to promote study and research in science."

Art. II. Section 1. (a)—from "upon payment of annual dues of $2.00 and upon nomination by two members" to "upon payment of annual dues of $3.00."
Section 1. (f)—from "($2.00)" to "($3.00)".
Section 2. Added: "Current membership dues are to be paid by May 15 in order to receive both numbers of the Journal. Members paying dues after that date will receive only the second number of the current volume."

Art. IV, Section 1—from "shall be elected for a two year period." to "and an alternate shall be appointed by the President for a two year period."
Section 2.—(Add this new section)—"The Academy representative to the A.A.A.S. Academy Conference shall be appointed by the President for a two year period."

By-Laws, 4.—from "accompanied by the initiation fee ($2.00)" to "accompanied by the annual dues ($3.00)."
6.—Added: "The Secretary-Treasurer shall receive an honorarium equivalent to 10% of the dues received during the current year."

(Note—At the first meeting of the new Executive Committee for 1954–55 on May 27, 1954 at Woman's College, U. N. C. it was voted to mimeograph the constitution as revised and make it available to any members who wished to have a copy.)

9. It voted to authorize the appointment of a special committee from the Academy consisting of the retiring President, the Secretary, and the new High School science chairman to be appointed to organize a larger Cooperative Committee to consider fundamental problems in the improvement of high school science in North Carolina. The larger committee will

include members from the North Carolina Department of Public Instruction, the North Carolina Section of the American Chemical Society, Duke University, University of North Carolina, North Carolina State College and Woman's College of the University of North Carolina. Among other projects which may be studied by such a committee it is recommended that regional science fairs be held in all six N.C.E.A. districts in the name of and to be sponsored by the Academy. Such fairs should be designed to increase the participation of both high school students and teachers in worthy scientific projects. These regional fairs should immediately precede a state-wide fair such as is held at our annual Academy meetings.

10. The committee accepted with pleasure the invitation of Davidson College to have the Academy meet there in 1955.

The above reports and actions of the Committee were approved by the Academy in its business session on May 7, 1954.

TREASURER'S REPORT

Financial Statement as of August 1, 1954

Receipts	
Checking account balance (from September 5, 1953)	$85.55
Gifts	
Ornithology essay award	20.00
N. C. Section, A.C.S.	10.00
Derieux award	50.00
Dues for 1954 (1953 and 1955)	1205.83
Total	1371.38
Expenditures	
Annual meeting expense, 1954	15.91
Postage, stationery, supplies	223.91
Printing	135.51
Bank charges	1.36
Secretarial aid	85.73
Secretary's commission	100.00
Expenses to representative to A.A.A.S. meeting	50.00
Awards (covered by special gifts)	70.00
Awards (given by the Academy)	80.00
Telephone (long distance)	14.89
Academy Conference	5.00
Reprints of Proceedings of the Academy	5.25
Elisha Mitchell Scientific Society	533.50
Total	1321.06
Bank balance, August 1, 1954	50.32
Total	1371.38

Savings Account

Balance (from September 5, 1953)	$106.12
Interest	2.12
	108.24

Total Assets

Checking account	50.32
Savings account	108.24
Raleigh Building and Loan Company	300.00
Total	458.56

On the twenty-eighth day of August, 1954, we, the undersigned, examined this account and found it to be correct.

(Signed) OWENS HAND BROWNE
BEN. W. SMITH
HARRY T. DAVIS, *Chairman*

THE POTEAT AWARD

The award for this year was given to the best paper presented before the Geology Section. The selection was made by members attending the section meeting of this group where papers were presented. The winner for this year is Maurice C. Powers of the University of North Carolina for his paper, "Clay mineralogical profile from fresh to salt water."

REPORT OF THE CONSERVATION AND LEGISLATIVE COMMITTEE

At the request of the President of the North Carolina Academy of Science, the Conservation and Legislative Committee distributed the "Resolution on State and National Parks" to North Carolina's Senators and Representatives, to the members of the Armed Services and Appropriations Committees in Congress, to the Chambers of Commerce in Raleigh and Durham, and to the North Carolina Department of Conservation and Development's Committee on Parks. This resolution, passed by the Academy on May 8th, 1953, was favorably received by the majority of the above agencies. The committee was informed that the resolution was of considerable assistance to those who successfully opposed the establishment of a carrier base at the Raleigh-Durham Airport.

The committee chairman, with the assistance of several other members of the Academy, devoted a number of days to an investigation of the terms of an exchange of land which involved approximately 217.5 acres of timbered park land for approximately 177 acres of farmland adjoining Crabtree Creek State Park. Many details of this transaction are still not clear, however. Adequate publicity has been given the matter by Raleigh newspapers to insure a thorough investigation by state officials.

The committee met in Raleigh on April 30, 1954, to discuss matters to be brought to the attention of the Academy and to take action on several items of business. As the members of the Academy are aware, the "initial phase" of the Upper Colorado Storage Project was recommended by the Secretary of the Interior and endorsed by the President of the United States. One of the proposed dams to be constructed in this "initial phase", Echo Park Dam, will be located in the Dinosaur National Monument and will inundate many miles of Green and Yampa Canyons in the heart of the monument. Since adequate alternate sites outside the monument are available, conservationists for many years have

consistently opposed Echo Park Dam as an unnecessary invasion of the National Park System.

For the first time in several decades, a Secretary of the Interior has openly recommended the violation of a National Monument. President Eisenhower, in a statement released Saturday, March 20th, threw the prestige and influence of his office behind the proposed Upper Colorado Basin project by specifically including in his recommendations the controversial Echo Park Dam. It is reliably reported that the White House Staff is referring all letters being received at the White House on this matter to the Department of the Interior. It is also reliably reported that of the messages received, nine to one object to the inclusion of the Echo Park Dam in H. R. 4449. The House Irrigation Subcommittee approved H. R. 4449 on May 3rd, and hearings will be held before the full Committee on Interior and Insular Affairs on May 13th. Both H. R. 4449, and companion bills H. R. 4443 and H. R. 4463, provide for the construction of the Echo Park Dam.

Your committee recognizes the need for irrigation and power in Western United States; however, it goes on record opposing H. R. 4449 as long as it includes authorization for the construction of Echo Park Dam. Alternate locations, equally advantageous, are available at the Gray Canyon and Cross Mountain Dam sites, which are outside the Monument.

The committee expressed its continued support of H. R. 1037, which would make the Dinosaur National Monument a National Park by Act of Congress.

The D'Ewart Bill, H. R. 4023, and the Butler Bill, S. 1491, often called the "Stockmen's Bills", were crushed by public outcry and have been returned to their respective committees for "burial". Senator Aiken's Bill, S. 2548, has passed the Senate; and Congressman Clifford R. Hope's companion bill, H. R. 6787, is being considered by the House Committee on Agriculture. The early versions of both bills have been amended in committee and would, in effect, accomplish almost the same favoritism of certain grazing interests as the discredited D'Ewart and Butler Bills. The Academy's attention is invited to the revealing article entitled, "It's Still Your Land", by Arthur Carhart, in the May 1954 issue of Sports Afield, a reprint of which is attached to the original copy of the committee's report.

Exotic species of game animals continue to be released in the State. Several hundred raccoons from Florida have been stocked in Western North Carolina. One positive rabid animal has been reported from this shipment.

A. F. CHESTNUT
WANDA S. HUNTER
T. E. MAKI
LAURA OWENS
COY T. PHILLIPS
F. S. BARKALOW, JR., *Chairman*

A Resolution on Use of Public Lands by the North Carolina Academy of Science, 1954

WHEREAS, the vast areas of public lands now administered by the U. S. Forest Service are suitable for such diverse uses as growing timber, producing wildlife,

grazing stock, supplying water to cities and industries, providing natural areas for scientific study, and furnishing recreation to millions of individual citizens; the management and administration of these lands in the best interests of all the people is a highly technical matter which can most efficiently be accomplished by flexible administrative procedures rather than by inflexible legislative directives; the U. S. Forest Service has demonstrated through the years its capability of handling these administrative responsibilities in a highly creditable manner; two bills now being considered by the Congress, S. 2548 and H. R. 6787, in their amended forms provide favorable provisions for only one small group of users of the public lands, possibly at the expense of part of all other groups of users; no provision is made for determination by economic survey whether the fees received from all permittees are equally just and are sufficient to insure the maintenance of these public lands; and the provision in these bills to permit trial by courts of disagreements based upon forage conditions arising from range use are likely to circumvent the wise administration and use of our public lands by delaying and preventing the removal of excess animals from an area until serious damage to the public range has occurred;

NOW, THEREFORE, BE IT RESOLVED that the North Carolina Academy of Science go on record as opposed to any legislation that grants special consideration to any special group of public land users at the expense of other legitimate users; and that the North Carolina Academy of Science is opposed to S. 2548 and H. R. 6787 in their amended forms, and that the President of the Academy is hereby directed to forward a copy of this resolution to North Carolina's Congressional delegation and to the House and Senate Committees on Agriculture.

The North Carolina Academy of Science unanimously approved this resolution at its annual business session on May 7, 1954.

HIGH SCHOOL CONTESTS WINNERS

Forestry Essay Contest

(*Sponsored by the North Carolina Forestry Association*)

"Better Tree Crops by Good Cutting Practices"

First Prize: Ann Hoyle, Henderson Junior High School, Henderson
Second Prize: Billy Culbreth, Route #4, Lumberton
Third Prize: Wiley Drye, Route #2, Salisbury

Ornithology Essay Contest

"Water Fowl Refuges in North Carolina"

First Prize: Carolyn Herring, Richlands High School, Richlands
Second Prize: Neil Andrews, Lumberton High School, Lumberton
Third Prize: Shirley Jean Cole, Cameron High School, Cameron

Exhibits Contests

Group I. Living Plants or Animals (*Sponsored by Carolina Biological Supply Co.*)
First Prize: Sally Lundlum, Tommy Thomas and Alton Millis, Lake Forest Junior High School, Wilmington

"Marine Aquarium Display"

Group II. Preserved Plants or Animals (*Sponsored by Carolina Biological Supply Co.*)

First Prize: Yvonne Westbrook, Grantham High School, Goldsboro
"Insect Collection"

Group III. Models

First Prize: Angus Duff, Greenville High School, Greenville
"Shaft Coal Mine Model"

Group IV. Miscellaneous

First Prize: Dwayne Lowder, Albemarle High School, Albemarle
"Water Purification System"

Group V. Chemistry Exhibits (*Sponsored by Cardinal Products, Inc.*)

First Prize: Jane Townsend, Myers Park High School, Charlotte
"Dyes from Nature"

Second Prize: Barbara Jinnette, Grantham High School, Goldsboro
"Analysis of 'nuts' of Nut-grass"

Winners of two honorary annual memberships in the American Association for the Advancement of Science were named from among the exhibit contestants. The winners were William Montgomery, Myers Park High School, Charlotte and Barbara Jinnette, Grantham High School, Goldsboro.

REPORT ON THE ACADEMY CONFERENCE

The 1953 meeting of the Academy Conference was held in Boston, Mass., on December 28, 1953 in the Hotel Bradford in connection with the meetings of the A.A.A.S. The attendance of thirty-seven official delegates out of thirty academies affiliated with the A.A.A.S. indicated the interest in the meeting. Eleven academies were not represented. The full minutes of the meeting are in the files of the Academy, but certain matters discussed there may be of interest to the members of the N. C. Academy.

Concerning the relation of the academies to the parent organization, the A.A.A.S., Mr. John A. Behnke, Associate Administrative Secretary of the A.A.A.S., announced that a new operational guide for all affiliated academies was in preparation. This guide has since been received by the Secretary. All academies are urged to appoint their delegates to the Academy Conference and to the A.A.A.S. Council on a calendar year basis, rather than on a school year basis.

Wayne Taylor of Texas becomes the President of the Academy Conference for 1954, while Leland H. Taylor of West Virginia was chosen as the new Secretary-Treasurer.

There was considerable discussion of the problem in connection with High School science. One motion was passed which requests the A.A.A.S. through its Co-operative Committee to study the proposal of accrediting science departments in the high schools of the country and to report its recommendations to the governors of the states and to the departments of public instruction in the states. The new President of the Conference was also instructed in one motion to institute a careful study of the Junior (High School) and Collegiate Academies

throughout the nation with a view to encouraging the organization of others in the states.

The second part of the Academy Conference consisted of round table discussions on the following four topics:

(1) Significant recent activities in the various academies.

(2) Opportunities of secretaries to serve their respective academies.

(3) Opportunities of academies to serve high school teachers.

(4) Opportunities of academies to serve the interest of the general public.

Appointed delegates to the Academy Conference concluded the meeting with a complimentary dinner furnished by the A.A.A.S. at the Hotel Bradford, at which a short concluding business session was held.

J. A. YARBROUGH
E. C. COCKE

REPORT ON THE AAAS COUNCIL

The Council of the A.A.A.S. met on December 27 and 30, in Boston, Mass. at the Hotel Statler. One hundred and eighteen members attended the first session, one hundred and twelve the second. Before the opening of the formal business session, Dr. Bronk, immediate past president, reported that the positions of Administrative Secretary and Editor had both been filled, the first position by Dr. Dail Wolfle, currently Director of the Commission on Human Resources and Advanced Training and formerly Executive Secretary of the American Psychological Association, and the editorship by Dr. Duane Roller, Assistant Director of Research, Hughes Research and Development Laboratories, California, and founder and former editor of the American Journal of Physics. In action later during the meeting, Dr. Meyerhoff and Mrs. Keener, who resigned from these positions in March of last year, were paid a special tribute for their services. Dr. Condon, presiding at this meeting, joined in adding his word of tribute and in regretting any misunderstandings that may have occurred. Thus ended a rather disturbing chapter in the life of the A.A.A.S. national office. At a later time Dr. Blackwelder of Washington, who had led the fight in defense of Dr. Meyerhoff, presented in a very mild way a motion that in the future the Administrative Secretary be responsible to the Board of Directors of the A.A.A.S. only and not to any particular individual. Dr. Weaver, the incoming president, seconded this motion, indicating, it would seem, full harmony on this point.

Dr. George W. Beadle, Professor of Biology and Chairman of the Division of Biology, California Institute of Technology, was duly elected president of the A.A.A.S. for 1955 and Dr. Wallace R. Brode and Dr. Thomas Park were chosen as new members of the Board of Directors for four year terms.

The Building Committee, seeking to build a national headquarters in Washington, is making a second appeal to the Washington Zoning Commission in order to obtain permission to construct the proposed structure. No success yet is reported.

Future meetings of the A.A.A.S. will be in 1954, Berkeley; 1955, Atlanta; 1956, New York; 1957, Indianapolis; and 1958, Washington, D. C.

At the first session on December 27 a special committee headed by Dr. Klop-

steg was appointed to frame a resolution reflecting the sentiment of the A.A.A.S. regarding President Eisenhower's recent statement concerning the beneficial utilization of nuclear energy. During the second session the following resolution was adopted, but only after repeated warnings from legal counsel of the Board of Directors that the Association should avoid any statement that might be interpreted as attempting to influence legislation, on pain of the possible loss of its tax exempt status; "Resolved, That the Council of the American Association for the Advancement of Science assembled at Boston on the occasion of its 120th meeting, expresses its approval and commendation of the proposal by President Eisenhower before the United Nations for international co-operation towards the beneficial utilization of nuclear energy. Many scientific and technical problems remain to be solved in such an undertaking. Scientists throughout the world will welcome the opportunity to work together on these problems as a service in the interest of peace and a contribution to the welfare of all peoples. Science is a major constructive force in the world. It knows no geographical boundaries. Hence the prospect of bringing scientists from many countries together in a collaborative research and development effort in this promising area provides great hope not only for immeasurable material benefits but especially for better understanding and good will among nations."

J. A. YARBROUGH

REPORT OF THE JOHN BEWLEY DERIEUX MEMORIAL AWARD COMMITTEE

Out of two papers submitted this year one was chosen unanimously by the committee to receive the award. The paper, "Theoretical effects of primary cosmic radiation on semiconductors above the atmosphere", was submitted by Charles B. Childs of the University of North Carolina. Mr. Childs also won the award in 1953. He was presented the award and certificate at the evening session of the Academy on May 7 by R. M. Helms of the committee.

R. M. HELMS
F. W. LANCASTER
GUITA MARBLE, *Chairman*

REPORT ON THE COLLEGIATE ACADEMY

The Collegiate Academy held its session for the reading of papers and organization of the group on May 7 at 2:00 p.m. A good attendance and a list of eleven papers indicate real interest in this new group within the N. C. Academy of Science. A committee from the Senior Academy heard and judged the papers. The Senior Academy presented two awards, one of $25 and one of $15, for the two best papers read at this session. The winners were: First place, Stephen Mahaley, Wake Forest College; Second place, Richard H. Newman, Elon College. Winning honorable mention were Joanna Woodson, Woman's College of U. N. C. and C. A. Edwards, Margaret McIntosh, and J. P. Rowland, East Carolina College. Stephen Mahaley and Richard H. Newman were designated to receive honorary membership in the A.A.A.S. for one year.

The Collegiate Academy elected the following officers: President, Joe P. Row-

land, East Carolina College; Vice-President, Edyth McCauley, Elon College; Secretary-Editor, Bill Owens, Atlantic Christian College. Dr. Charlotte Dawley, Woman's College of U. N. C. was designated as advisor for the Collegiate Academy by President Puckett of the Senior Academy. The officers, the advisor and the Secretary of the Senior Academy will constitute an executive Committee to plan the program for the 1955 meeting.

JOHN A. YARBROUGH

REPORT OF THE RESOLUTIONS COMMITTEE

Be it resolved that the North Carolina Academy of Science express to President J. D. Messick, the other administrative officers, the faculty, and the students of East Carolina College its gratitude for the hospitality and many services that contributed to the success of the Academy's fifty-first meeting. Be it further resolved that the Academy convey its great appreciation to the members of the local Committee on Arrangements for their role in planning for this meeting. Be it further resolved that a copy of this resolution be incorporated into the minutes of the Academy and that copies be sent to President Messick and to the chairman of the Committee on Arrangements.

EVA R. BALKEN

The above resolution was unanimously approved by the Academy.

Memorials to the following members were read before the Academy: Stephen Cole Bruner and William Chambers Coker. Each memorial was approved by a rising vote.

STEPHEN COLE BRUNER

Stephen Cole Bruner was born in Raleigh, North Carolina, on August 18, 1891 and died in Loma Linda, California, August 27, 1953. He was the son of Thomas K. and Belle Boyden Bruner. He was educated in the Raleigh schools and graduated from the Raleigh High School in 1908. He attended North Carolina A. and M. College, now North Carolina State College, from 1908 to 1912 and was graduated with the degree of B.S.

He was employed by the United States Department of Agriculture from 1912 to 1914. In 1915 he became assistant pathologist of the Estacion Experimental Agronomica in Cuba, pathologist in 1918, and he was chief of the Department of Plant Pathology and Entomology from 1919 until his death.

Dr. Bruner was actively interested in natural history from his earliest childhood. He was chiefly influenced by his associations with C. S. Brimley, naturalist, and H. H. Brimley, curator of the State Museum, and Franklin Sherman, the state entomologist. He never lost his interest in ornithology and in later life he specialized chiefly in the taxonomy of the hemipterous insects and in the economic entomology of Cuba.

For his achievements as a scientist and for his contributions to tropical agriculture, Dr. Bruner was awarded the honorary degree of Doctor of Science by North Carolina State College in 1939. In October, 1950 he was awarded the rank

of Knight of the National Order of Merit "Carlos Manuel de Céspedes" for his long and distinguished service to the Cuban government. The Cuban government also conferred upon him the Order of Merit in Agriculture and Industry.

Dr. Bruner's bibliography is very extensive. It includes sixty papers on entomology, twenty-three on plant pathology, mycology, etc., nineteen on ornithology, and many other articles on agriculture. During the thirty-eight years that Dr. Bruner lived in Cuba he filled many positions of responsibility. In his lifetime he was honored by having thirty-one species of insects named after him.

Z. P. METCALF

WILLIAM CHAMBERS COKER

William Chambers Coker was born on October 24, 1872 in Hartsville, South Carolina, and died at his home in Chapel Hill, North Carolina on June 27, 1953.

His early education consisted of home tutoring, largely by a governess. Later he attended a small college-preparatory school in Hartsville. In 1891 he entered the University of South Carolina and was graduated in 1894.

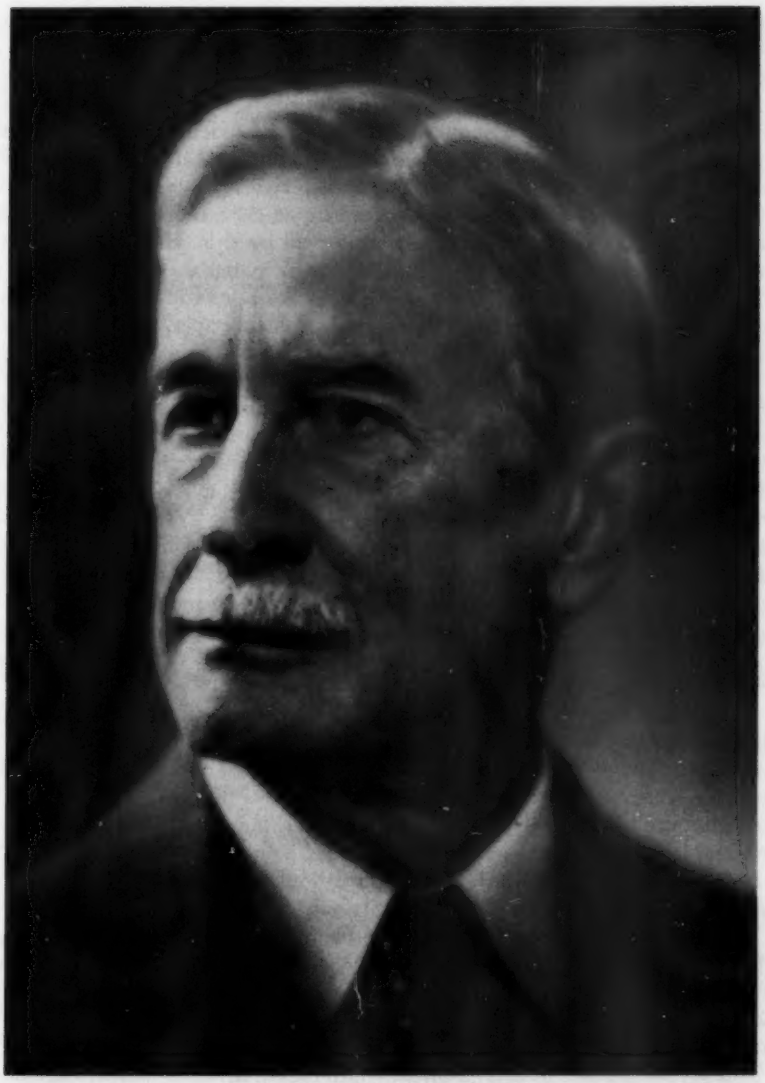
The year of his graduation he began working in the Atlantic National Bank at Wilmington, North Carolina, becoming one of the vice-presidents two years later. The next year, 1897, he decided to give up banking and to do graduate work in botany at the Johns Hopkins University. In this decision he received the whole-hearted support and encouragement of his family. After receiving the Ph.D. in 1901 from Johns Hopkins, he spent a semester in Strasburger's laboratory at Bonn-am-Rhine, Germany.

In the fall of 1902 Dr. Coker joined the faculty of the University of North Carolina as associate professor of botany, and served continuously until his retirement in 1945 as Kenan Research Professor, Emeritus.

Dr. Coker was first and foremost a productive botanist. During a period of about fifty-one years, one hundred and thirty-seven publications appeared. Seven of these were books and the others ranged from long monographs to abstracts. Although covering diverse fields from algae to seed plants, his most important publications were on fungi. In spite of his many and excellent contributions on the higher fungi, his fame as a mycologist rests largely on his publications on the water fungi, which have had wide influence in stimulating researches in this field. His eminence as a mycologist and botanist brought him many honors at home and abroad.

Dr. Coker was also an authority on the taxonomy of the woody plants. In 1916 "The Trees of North Carolina" was published with H. R. Totten, and under their joint authorship later was extended to cover the Southeastern States. The first edition of the expanded book was published in 1934. The latter is now in the third edition and second printing.

For many years Dr. Coker's major interests in teaching were devoted to the course in general botany, which seems to have been modelled much after Dr. Johnson's course at Hopkins. As an undergraduate teacher he ranked high among the faculty, although his methods were sometimes not wholly conventional. In his general botany classes he began with the low forms of plant life and usually



WILLIAM CHAMBERS COKER

progressed to the more advanced types; the exact procedure, however, was never twice the same. A walk from his home through the village and arboretum might suggest an entirely new topic for that day's discussion, and the assistant who had prepared the classroom demonstrations could never foretell when a complete

change might have to be made in the last few minutes before the lecture began. But the lecture fitted the time and caught the students' interest. As a teacher of graduate students and director of research, he was superb.

Dr. Coker was president and director of the Biological Laboratory at Highlands, North Carolina, for a number of years. He did much to carry the laboratory through its early years and was largely instrumental in developing it into an important biological station.

Dr. Coker joined the North Carolina Academy in 1902, the year it held its first annual meeting, and two of his papers were read by title. He seldom missed a meeting, and usually, when in attendance, participated in the programs. His presidential address before the Academy at Wake Forest in 1910 on "Science Teaching in the High Schools" was a very thoughtful paper, and is still timely and interesting reading. In 1904 The Journal of the Elisha Mitchell Scientific Society was made the official publication of the North Carolina Academy of Science and Dr. Coker was the chairman of the editorial committee from this time until his retirement in 1945.

The achievements of Dr. Coker most easily seen and appreciated by non-botanists were in landscape architecture. This remarkable natural talent, improved by study and practice, resulted in the development of the Coker Arboretum and the simple, informal plantings on the campus at Chapel Hill. He gave advice and plants freely to private citizens who wished to beautify their home grounds. His practical interest in the use of plants, especially native plants, resulted in the publication of two bulletins on landscape design in the early twenties and in the planting of shrubs, under his direction, on many of the school grounds throughout the state.

For over fifty years, Dr. Coker was one of the most striking figures in Chapel Hill. He was tall and slender and had unusual personal charm. He was in every sense a gentleman—dignified but friendly, calm and reserved but sensitive to the needs of others, often quietly opening up avenues of financial assistance to struggling students. He helped many young men through college by making them personal loans. Though he lost money on some, he never lost faith in people. He was fond of animals, and although he always chose a mongrel for his dog, the dog assumed the aristocratic dignity of its master.

Dr. Coker remained a bachelor until October 28, 1934, when he married Louise Manning Venable, daughter of President Venable, and her companionship and affection filled the one void in his life. Their home became a mecca for neighbours, friends, and a multitude of devoted nephews and nieces. Their talk and laughter amused and delighted the genial host, crowning a full and fruitful life with a joyous old age. Finally, as his strength ebbed, so serene were his surroundings and so gentle his care, that his span of life was stretched to four score years.

In his death the University has lost one of her most distinguished professors and the Academy a devoted and loyal member.

H. R. TOTTEN
J. N. COUCH

REPORT OF THE NOMINATING COMMITTEE

The committee, consisting of C. F. Korstian, chairman, H. R. Totten and P. M. Ginnings, submitted the following nominations:

President: W. O. Puckett, Davidson College
Vice-President: A. D. Shaftesbury, Woman's College of U. N. C.
Member of the Executive Committee: C. S. Black, Wake Forest College
Member of the Research Grants Committee: Maurice Whittinghill, U. N. C. (3 years)

The above nominees were unanimously elected by the Academy. This concluded the business and the meeting was adjourned.

Approximately 125 members of the Academy and friends enjoyed the Annual Dinner of the Academy which was held in the Dining Hall on the East Carolina College campus at 6:15 p.m. At 8:00 p.m. the evening session was held in the Flanagan Building with Vice-President Florence L. Schaeffer presiding. The 1953 Poteat Award was presented to Dr. Elizabeth K. Goldie-Smith of the University of North Carolina and the 1954 John Bewley Derieux Memorial Award to Charles B. Childs of the University of North Carolina. Miss Mary O. Ellington announced the winners of the High School Essay Contests. The interesting address of President Donald B. Anderson was enjoyed by the membership and guests.

The following officers were elected for the respective sections:
Biochemistry and Physiology: Chairman, E. P. Hiatt; Secretary, G. H. Satterfield
Botany: Chairman, Arthur Kelman; Secretary, Ben W. Smith
Geology: Chairman, Richard J. Councill; Secretary, E. W. Berry
Mathematics: Chairman, Whitfield Cobb; Secretary, W. N. Mebane
Zoology: Chairman, Charles E. Jenner; Secretary, Walter M. Kulash

The following constitute the personnel of the standing committees:
Executive: W. O. Puckett, A. D. Shaftesbury, John A. Yarbrough, Eva G. Campbell, E. W. Berry and Charles S. Black
Research Grants: L. E. Anderson, H. E. LeGrand, O. J. Thies, Maurice Whittinghill

Dr. W. O. Puckett, President of the Academy for 1955, announced the appointment of the following committees:
Auditing: Harry T. Davis, chairman; O. H. Browne, T. E. Maki
Nominating: H. R. Totten, chairman; B. W. Wells, F. A. Wolf
High School: Hollis T. Rogers, chairman; H. A. Shannon, Mazel B. Lyke, J. O. Derrick, Raymond Hatley
Conservation-Legislative: F. S. Barkalow, chairman; T. E. Maki, Dr. Wanda Hunter, Coy T. Phillips
Resolutions: John M. Flowers
Derieux Memorial Award: R. M. Helms, chairman; J. S. Meares, O. H. Browne

Representative on A.A.A.S. Council: John A. Yarbrough
Representative to Academy Conference: John A. Yarbrough

The following papers were presented during the meetings. Those marked with an x are abstracted in these proceedings:

Presidential Address: The Structure of Plant Cell Walls. Donald B. Anderson, North Carolina State College

SYMPOSIUM ON GROWTH AND DEVELOPMENT

The role of apical meristems in primary growth of plants. Ernest Ball. North Carolina State College

Localization and differentiation in the animal egg, as exemplified by the early development of the annelid Nereis. D. P. Costello, University of North Carolina

The conditionality of the initiation of neoplastic change and the subsequent growth of neoplastic cells. Stanfield Rogers, Duke University School of Medicine

The form problem, "Growth requirement versus growth regulation". Victor M. Cutter, Jr., Woman's College of the University of North Carolina

COLLEGIATE ACADEMY

Insectivorous plants. Thomas D. Kitchen, Wake Forest College

An ecological study of the college campus. Doris Dunn, Flora Macdonald College

Experiments on the isolation in pure culture of the green algal component of Hydra viridissima. Joanna Woodson, Woman's College U. N. C.

Mass cultures of Chlorella as food sources. Betty Vance, Meredith College

The gasification of coal. D. C. Dahl, Duke University

x*Observation on temperatures of hibernating bats.* Stephen Mahaley, Wake Forest College

Special adaptations of the angler fish. Charles A. Edwards, Margaret Ann McIntosh and Joe P. Rowland, East Carolina College

Some notes on filter paper electrophoretic separation of amino acid mixtures. Charles W. Phillips, Jr., Elon College

x*Some applications of paper chromatography to inorganic qualitative analysis.* Richard H. Newman, Elon College

Studies related to tert-octylphenol. D. York Brannock, Jr., Elon College

Studies related to allylmalonic ester. Philip R. Mann, Elon College

BIOCHEMISTRY-PHYSIOLOGY SECTION

Studies on the growth response of chicks to antibiotics. C. H. Hill and J. W. Kelly, N. C. State

x*A study of fatty acid oxidation in tissues of rats exposed to intermittent periods of hypoxia and in tumor tissue of mice.* B. Theodore Cole, Duke University School of Medicine

Action of dietary cystine on the oxidation and synthesis of fatty acids. Camillo Artom, Bowman Gray

x*Depolymerization of desoxyribonucleic acid preparations by oxidized methyl linolenate.* Wm. D. Fisher and Karl M. Wilbur, Duke University

A statistical problem in biochemistry. Robert J. Monroe, N. C. State

Non-oxidative exchange between inorganic and nucleotide phosphate. Marjorie Swanson, Bowman Gray

x*Studies of the mechanism of the formation of renal calculi.* James C. Andrews and Claude L. Yarbro, University of North Carolina School of Medicine

In vivo production of trimethylamine from choline. Camillo Artom and H. B. Lofland, Bowman Gray

x*Composition of browse herbage and seasonal changes in the Holly Shelter game refuge.* Frank Smith, N. C. State

BOTANY SECTION

x*Changes in bioelectrical potentials associated with surface injuries in fruits.* Otto Stuhlman, Jr., U. N. C.

Range extensions of some North Carolina plants. Lionel Melvin, Pleasant Garden. (Printed in full elsewhere in this issue of the Journal.)

x*A differential medium for detection of mutants of the Granville wilt bacterium.* Arthur Kelman, N. C. State

The nature and relationships of Scenomyces perplexans Stevens. J. N. Couch, U. N. C.

Algae of the giant springs of Florida. L. A. Whitford, N. C. State

Chromosome studies of some mosses of the Southeastern United States. Virginia S. Bryan, Duke

Chromosome behavior in a moss, Clasmatodon parvulus (Hampe) Sull. Lewis E. Anderson and Virginia S. Bryan, Duke

x*Nuclear aberrations in the syncytial endosperm of Cocos nucifera.* Bessie Freeman and V. M. Cutter, Jr., Woman's College, U. N. C.

x*Reciprocal grafts between genetically lethal and normal cotton.* J. B. Weaver, Jr., N. C. State

An attempt to trace the path of conduction through the non-vascular tissues of Ficus leaves. Jane Philpott, Duke

GEOLOGY SECTION

x*Preliminary study of flood-plain deposits in North Carolina.* R. G. Schipf and H. E. LeGrand, U. S. Geological Survey, Raleigh

x*Deposits of middle Miocene age in Martin County, North Carolina.* P. M. Brown, U. S. Geological Survey, Raleigh

x*A dome of brackish ground water in the coastal plain of North Carolina.* H. E. LeGrand, U. S. Geological Survey, Raleigh and J. R. LeGrand, Atlantic Coast Line Railroad

x*Clay mineralogical profile from fresh to salt water.* Maurice C. Powers, U. N. C.

x*Clay minerals and paleoclimatology.* Roy L. Ingram, U. N. C.

x*The adjustments of land-derived clays to the Neuse River estuary.* George M. Griffin and Roy L. Ingram, U. N. C.

x*Geology in relation to the seven life zones of biology and the seven language zones of ethnology.* A. L. Pickens, Queens College

Some problems encountered in surveying small diamond drill holes. Duncan Heron, Duke

x*Geology of northeast Angola.* E. W. Berry, Duke

x*New developments in titanium.* Edward C. Nixon and C. Edward Howard, N. C. State

MATHEMATICS SECTION

Comments on the teaching of trigonometry. T. F. Hickerson, U. N. C. (Printed in full elsewhere in this issue of the Journal.)

Group teaching in plane geometry. John F. Williams, Myers Park High School, Charlotte

A method of presenting the vertex of a parabola in college algebra. Anne Lewis, Woman's College, U. N. C.

A mathematics course for high school teachers. A. K. Hinds, Western Carolina College

What is a point? F. Burton Jones, U. N. C.

ZOOLOGY SECTION

History and distribution of the carrot rust fly. H. Eldon Scott, N. C. State

Origin of stored food pests. Walter M. Kulash, N. C. State

Fecundity studies as an approach to life history problems in fishes. William E. Fahy, U. N. C.

Photoperiodism in the mosquito fish, Gambusia. Charles E. Jenner, U. N. C.

x*The amphibians of New Hanover County.* John B. Funderburg, Jr., East Carolina College

x*The population structure of the spiders of the abstract broomsedge community in the Piedmont of Southeastern United States.* Robert D. and Betty Martin Barnes, Duke

A study of embryonic segregation in Ilyanassa obsoleta. I. The distribution of alanylglycine dipeptidase activity in the early blastomeres and germinal regions. Jack Collier, U. N. C.

Studies of gene linkage after irradiation. Maurice Whittinghill, U. N. C.

100 years of professional entomology. Clyde F. Smith, N. C. State

Experimental Evidence on the Body Temperatures of Hibernating Bats. M. S. MAHALEY, JR.

Observations of bat populations in the caves of North Carolina during the winter months have provided evidence concerning the hibernating tendencies of several species. The observations recorded in this paper are based upon the direct effect on body temperature of the metabolic rate fluctuations between hibernation and active body functioning. The experiments were carried out on January 2, 1954; the temperature outside the cave was 48° F. The measurements of body temperatures were made on the following species of hibernating bats: *Myotis*

keeni and *Pipistrellus subflavus*. The instrument used in obtaining the body temperatures of the bats was a thermistor connected to a Wheatstone bridge. Although the experimentation has not been very extensive, the evidences seem to indicate several interesting facts. The differences found to exist between the body temperatures of the separate species and the different sexes of the same species may be significant. A more obvious characteristic, especially in the case of the species *Pipistrellus subflavus*, is a lowering of the body temperature during hibernation to a level near that of the environment (46° F). The increase in body temperature from that of hibernation to a comparatively active condition was 14.4° F in the case of the individual of the species *Myotis keeni*. The facts that the bat's temperature drops during hibernation to a temperature comparable to that of the environment, and that this hibernation temperature of the bat increases considerably when the animal assumes activity, indicate poikilothermic functioning of the nervous system of the bat similar to that which exists in lower vertebrate groups.

Some Applications of Paper Chromatography to Inorganic Qualitative Analysis. RICHARD H. NEWMAN.

An attempt was made to apply the methods of paper chromatography to the separation and identification of the cations within two of the groups of the non-hydrogen sulfide scheme given by West, Vick and LeRosen in their text *Qualitative Analysis and Analytical Chemical Separations.*

The equipment used in the experimental work consisted of a test tube approximately 35 cm. long and 2.5 cm. wide. The tube was fitted with a one-hole bung into which was inserted a glass rod which had been drawn out and bent into the shape of a hook. A strip of Whatman No. 1 paper, one-half inch wide and approximately 30 cm. long, was used as the stationary phase.

The test spot was placed on the paper with a capillary tube; after the spot had dried, the strip was suspended from the hook inside the tube in such a way that the lower end of the strip was just above the surface of a few ml. of solvent which had been placed in the bottom of the tube. After remaining this way for 30 min. the end of the strip was lowered into the solvent for a matter of hours until the solvent had crept up to the end of the paper. The strip was removed, dried, and sprayed with a test solution in order to determine the position to which the ion or ions from the original test spot had migrated. Reclaimed deodorant bottles were used for spraying.

Ions of group I were successfully separated and identified. Identification of most of the ions of group II has been effected in separate tests; separation of these ions is still under study.

Tests made, solvent and test solutions used, are shown in the table on the following page.

Group I	*Solvent*	*Test Solution*
Ag'	70% sec-butyl alcohol	Na_2S
Hg_2''	70% sec-butyl alcohol	Na_2S
Ag' & Hg_2''	70% sec-butyl alcohol	Na_2S

Group II	*Solvent*	*Test Solution*
Fe'''	70% sec-butyl alcohol	KCNS
Cr'''	70% sec-butyl alcohol	Alizarin
Al'''	70% sec-butyl alcohol	Alizarin
Bi'''	70% sec-butyl alcohol	$SnCl_2$
Sb'''	70% sec-butyl alcohol	Na_2S
Sn''''	70% sec-butyl alcohol	no satisfactory reagent as yet

A Study of Fatty Acid Oxidation in Tissues of Rats Exposed to Intermittent Periods of Hypoxia and in Tumor Tissue of Mice. B. THEODORE COLE.

The work of Kohn and Liversedge (1944); Bernheim, Bernheim and Wilbur (1948); Wilbur, Bernheim and Shapiro (1949) all give substantial evidence that the oxidative products of certain unsaturated fatty acids can be determined colorimetrically. The latter group prove conclusively that the methyl ester of linolenic acid after autoxidation or oxidation catalyzed by ascorbic acid yields a compound which reacts with thiobarbituric acid (TBA) to produce a colored compound with a characteristic absorption spectra.

TBA values for "bound" fraction, unincubated testes tissue of rats exposed to discontinuous periods of hypoxia and thus made sterile, are lower than values for tissue of control animals. Values for the "bound" fraction of incubated tissues are likewise lower in the experimental group. Values for the "unbound" fraction of tissues incubated with ascorbic acid reflect no statistically significant difference in the quantity of linolenic acid available for oxidation in the two groups, whereas the increased value for "unbound" fraction, experimental tissue, incubated without ascorbic acid, is highly significant.

TBA values reflect a greater amount of linolenic acid available for oxidation in lung tissue containing adenomas than in normal lung tissues of mice. High TBA values indicate that the growing portion of mice breast tumor contains larger amounts of linolenic acid than does medullary (necrotic) tissue of the same tumor. The TBA values reflect this greater concentration whether or not the tissues be incubated with ascorbic acid. This is a corollary of the fact that TBA values for medullary tissues incubated with ascorbic acid do not reflect the catalytic action of ascorbic acid on the reaction involved.

Depolymerization of Desoxyribonucleic Acid Preparations by Oxidized Methyl Linolenate. WILLIAM D. FISHER AND KARL M. WILBUR.

The biological effects of radiation have been thought to involve the production of organic peroxides. The depolymerization of desoxyribonucleic acid (DNA) is one of the well-known effects of both ionizing and ultraviolet irradiation. Since unsaturated fatty acids are oxidized by radiation and could provide a source of peroxide, the action of irradiated fatty acids on DNA has been studied.

Purified methyl esters of linoleic and linolenic acids oxidized to various degrees by ultraviolet light were added to a highly polymerized DNA preparation (Anderson, Nature **172:** 807, 1953) of rat spleen and the depolymerization followed in a modified Ostwald viscometer. Oxidized methyl linolenate caused a

marked decrease in the viscosity of the DNA preparation, whereas unoxidized methyl linolenate was without effect. Methyl linoleate of the same peroxide value as oxidized methyl linolenate samples was ineffective.

The effect of oxidized linolenate indicates a possible indirect depolymerization of DNA by ultraviolet light as a result of oxidation of unsaturated fatty acids in addition to the well-known direct depolymerization by ultraviolet light. Since lipids and DNA are both present in the nucleus, the present findings also raise the question whether factors which normally alter the degree of oxidation of fatty acids in the cell have an effect on DNA.

*Studies of the Mechanism of the Formation of Renal Calculi**. JAMES C. ANDREWS AND CLAUDE L. YARBRO.

The frequency with which renal calculi are encountered, particularly in certain specific areas, has been noted clinically for many years. An example of one of these areas with a high incidence of such calculi is the central part of North Carolina. However, attempts to determine the cause of this higher incidence have met with no success. The majority of these calculi consist of calcium phosphate and calcium oxalate, usually mixed.

The authors suggest that there may be very great differences in the intrinsic abilities of different individuals to absorb calcium and that an unusually great absorptive ability may well be the cause of an habitually higher output of urinary calcium resulting in a correspondingly greater tendency to form calcium stones. A quantitative comparison of calcium absorption by normal subjects compared to that by habitual "stone formers" is being planned.

The present report concerns the equilibrium conditions found in the urine of both classes of individuals. To determine if these urines are unsaturated, saturated or supersaturated with respect to both calcium phosphate (secondary or tertiary) and calcium oxalate, the following procedure was employed: In the urine, as passed, there were determined the pH and the content of calcium, magnesium, phosphate, oxalate and urate. Another portion of the same urine was then shaken mechanically with a small added amount of either solid dicalcium phosphate, tricalcium phosphate or calcium oxalate until equilibrium was established (12 to 18 hours). The above analyses were then repeated. In practically all cases where tricalcium phosphate or calcium oxalate was used a very definite decrease was observed, thus indicating that the urine was to be regarded as an originally supersaturated solution from which deposition of solid calculi in normal subjects was merely sufficiently delayed. This delay is obviously increased because of the presence of urinary colloids. No difference in behavior, however, could be detected between the urines of normal subjects and those of habitual stone formers.

Similar equilibration studies have been reported by Flocks. However his technique consisted in shaking by hand the urine samples with a small amount of the solid phase for about 10 minutes—in the author's opinion, a procedure very un-

* Supported by a grant-in-aid from the U. S. Public Health Service.

likely to result in true equilibrium. To test this point, we have taken various samples of the same urine and have varied the time of mechanical shaking from 10 minutes to 18 hours, determining, at each interval of time, the phosphate and the pH. We find that when the solid phase used is tricalcium phosphate there is a sharp drop in the dissolved phosphate at about 10 minutes' shaking, followed by a sharp rise to a plateau value somewhat lower than that of the original urine. The extent of this drop varied somewhat with different samples of urine. Instead of a similar decrease, an increase was observed when secondary calcium phosphate was used as the solid phase. The work is being continued.

Composition of Browse Herbage and its Seasonal Changes in the Holly Shelter Game Refuge. F. H. SMITH.

This study was undertaken to determine the adequacy of the herbage in supplying the required nutrients of deer in the Holly Shelter Refuge. Five plants (*Smilax laurifolium*, *Ilex glabra*, *Cyrilla racemiflora*, *Myrica ceriferi*, and *Vaccinium crassifolium*) comprising the major ingredients of the diet of deer in this area were analyzed for the nutrients required by the animals.

The most striking information obtained from this study was the extremely low phosphorus content of these plants, except in the spring when the plants were making rapid growth. It is possible that the phosphorus requirements of ruminants are met during the spring period of lush growth but are somewhat less than allowances recommended for sheep. During the other nine months of the year, these plants supply only about one fourth of the amount of phosphorus recommended for sheep.

The protein content of these plants followed the same pattern as the phosphorus. It was high and undoubtedly adequate in the spring when the tender young shoots and leaves were abundant. For the remainder of the year it was too low to supply adequately the protein required.

Other nutrients present in the herbage of the Holly Shelter area were found in adequate amounts, with one exception, if utilized efficiently. Cobalt was low and relatively constant throughout the year at about 0.05 p.p.m. This level is in the deficiency range. The crude fiber content was unusually low in the spring and was low for the remainder of the year. It was markedly lower than that for alfalfa hay. The nitrogen-free extract was higher than that found in most forage crops and was relatively constant throughout the year. Calcium was lowest in spring and highest in fall and winter. Similar studies in the mountain area showed that the forage contained sufficient nutrients to meet more adequately the requirements of livestock.

It is postulated from this study that the main problem in the Holly Shelter area is inadequate phosphorus, cobalt, and marginal levels of protein in the herbage comprising the diet of the deer. A supplementation of the forage with phosphorus and cobalt at feeding stations should greatly improve the nutrition of the deer. A further improvement could be expected if some means were found to keep rapidly growing herbage over a longer period of the year; this could be enhanced by providing protein concentrate during the winter months.

Changes in Bioelectrical Potentials Associated with Surface Injuries in Fruit. OTTO STUHLMAN, JR.

A repetition of Beutner's experiments on electrical potential changes over the surfaces of apples due to pressure injuries verified the observation that the injured surface develops a negative potential relative to any uninjured area.

The bioelectrical potential gradients were measured with a modified form of vacuum tube microvoltmeter as used by Burr, Lane, and Nims (Yale Jour. of Biol. and Med. **9:** 65, 1936).

Equatorial sections through symmetrical apples were explored for equipotential surfaces, without much success. Regions of maximum rate of growth were found to be electropositive to regions of lower rates of growth, in agreement with the observations of Ramshorn (Planta **22:** 737, 1934) later verified by Lund. Explorations on the changes in electrical potential over the surfaces of uninjured bananas showed that the brown areas, developed during ripening, are "injuries" and are electronegative to yellow skin areas. Injuries due to local pressures, without immediate discolorations, were always electronegative. Discolorations accompanying ripening are probably old pressure areas or ridges.

High electro-negative values due to pressure recover nearly logarithmically, with time.

A Differential Medium for the Detection of Mutants of the Granville Wilt Bacterium. ARTHUR KELMAN.

In cultures of *Pseudomonas solanacearum*, causal agent of Granville wilt of tobacco, mutants arise which form colonies differing in morphology from those of the normal or wild type. The wild colony type is usually irregularly round, fluidal and opaque. The mutant colony type found most frequently is uniformly round, non-fluidal or butyrous, and transparent. In both types the colony surface is smooth. Mutants which form rough non-fluidal and transparent colonies have been found infrequently. The presence of mutant colonies can usually be determined by careful examination of streaked cultures with obliquely transmitted light under a dissecting microscope. However, detection of mutant colonies is greatly facilitated by streaking dilute bacterial suspensions on a glucose-peptone-casein hydrolysate medium containing 0.005% triphenyl tetrazolium chloride. After 36 hours of growth at 32°C., colonies of the wild type are either entirely white or white with a small light red center. In contrast, colonies of mutants appear dark red with a narrow white border.

Use of a medium containing tetrazolium chloride has facilitated the study of (1) relative percentages of normal and mutant types in stock cultures, and (2) population changes following growth in inoculated host plants and in various media.

In addition, initial experiments indicate that a relationship exists between colony pigmentation and pathogenicity. Mutants which form dark red colonies with a narrow light border on the tetrazolium chloride medium have been found to be less pathogenic than the wild type, colonies of which do not show this intense coloration.

Nuclear Aberrations in the Syncytial Endosperm of Cocos nucifera. BESSIE FREEMAN AND V. M. CUTTER, JR.

In fruits of *Cocos nucifera* about 50 mm. in length the embryo sac is filled with a watery fluid (milk) in which large free nuclei and some cytoplasmic particles, but no discrete cells, are suspended. At a somewhat later stage (150 mm.) these free nuclei act as centers around which the protoplasm coalesces to form large spherical cells or endosperm vesicles which also float free in this liquid syncytium. The nuclei undergo division while suspended in the syncytium both before and after their incorporation into vesicles, yet in neither case has any evidence been detected of the occurrence of mitoses or the formation of any type of achromatic figure. The nuclear aberrancies are not confined to any one type and certain figures very closely resemble metaphase plates, but the apparent absence of prophase stages and spindle fibers in these cases as well would seem to give support to the theory of Darlington (1937) that, at least in the case of *Cocos*, "Amitosis is characteristic of the short lived endosperm of seed plants." The various types of amitotic division figures encountered in the free nuclei and endosperm vesicles of *Cocos* were described and illustrated.

Reciprocal Grafts Between Genetically Lethal and Normal Cotton. J. B. WEAVER, JR.

Hybrids between tetraploids of the Sanguineum type of *Gossypium arboreum* and *G. hirsutum* (a natural tetraploid) are lethal, owing to the interaction of genes from the two species, as has been shown previously. Intensification of red pigmentation ("red lethal") and bending down of petioles are followed by death at about the onset of the flowering stage. Reciprocal grafts between the genetically lethal hybrids and the Coker variety of *G. hirsutum* were made to determine the cause of death of these hybrids. Red lethal hybrid scions were successfully grafted upon eight Coker stocks in the seedling stage. All of these lethal scions showed the same reddish discoloration and drooping of the leaves, as did ungrafted lethal control plants on their lethal roots. From this result it was concluded that death of the lethals was not due to abnormal mineral nutrition.

Nine Coker scions grafted on lethal hybrid roots did not show any of the characteristic symptoms of the lethals but instead they wilted and apparently died of starvation two months after grafting. Thus it seems that abnormal photosynthesis is not the cause of death of the lethals either, since the normal Coker shoots could not make the lethal hybrid roots live. A brown discoloration, typical of the lethal type, was found in the xylem of the lethal hybrid stock but not in the Coker scion even within the graft region. Apparently the cause of discoloration is not a diffusible poison. It is concluded that death occurs autonomously in any plant organ carrying the lethal gene combination.

Preliminary Study of Flood-Plain Deposits in North Carolina. R. G. SCHIPF AND H. E. LEGRAND.[1]

An investigation of flood-plain deposits as a source of ground-water supply in North Carolina is being made by the United States Geological Survey in co-

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operation with the Division of Mineral Resources, North Carolina Department of Conservation and Development. Few wells have penetrated these alluvial deposits. Their distribution and water-bearing character are, therefore, little known. To learn the potential value of this ground-water supply, it has become necessary to undertake a critical study of the factors controlling flood-plain deposition, especially in the Piedmont and Mountain sections.

An appraisal of data from the few existing wells and from 67 test holes mechanically augered in these deposits during 1953 indicates that the genetic classification of flood-plain deposits developed by Happ, Rittenhouse, and Dobson is pertinent. The significant types are: (1) channel-lag deposits, composed of relatively coarse materials, which intermittently shift in the beds of streams until they become covered by (2) lateral-accretion deposits, which generally are sandy deposits formed on the inside of channel bends as the channel migrates laterally. These are generally covered by (3)vertical-accretion deposits, representing poorly sorted material deposited during floods.

The streams in the Piedmont and Mountain sections of North Carolina are almost everywhere degrading their channels; consequently, alluvial deposits extending deeper than 5 feet below the level of the channel bottom are uncommon. In spite of the relative thinness of the saturated alluvium, the development of large ground-water supplies is possible from buried lateral-accretion and channel-lag deposits, especially from recently deposited lateral accretions on the insides of channel bends.

Deposits of Middle Miocene Age in Martin County, North Carolina. P. M. BROWN.[2]

Recent ground-water studies, being made by the United States Geological Survey in co-operation with the Division of Mineral Resources, North Carolina Department of Conservation and Development, have revealed the presence of heretofore undescribed deposits of middle Miocene age at a horizon approximately 30 feet below sea level at Jamesville, Martin County. These deposits, grayish-green arenaceous shell marls containing abundant Ostracoda and Foraminifera, are thinly blanketed by the Yorktown formation of late Miocene age and by Pleistocene material.

On the basis of Swain's description of Cenozoic Ostracoda from wells in North Carolina, it is suggested that the ostracodes in the present material could range from middle to upper Miocene. However, none of the species restricted by Swain to the Upper Miocene occur at Jamesville. Three species of ostracodes, restricted by Malkin to the Choptank and Calvert formations of Maryland and Virginia, are present in the material at Jamesville—*Trachylibris exanthamata*, *Haplocytheridea subovata*, and *Cytheretta inaequivalis*. These and the Foraminifera *Lagena acuticostata*, *Quinqueloculina contorta*, and *Quinqueloculina* cf. *Q. fusca* suggest a Choptank to Calvert age for the deposits. Affinity with the *Arca* and *Ecphora* zones of the Choctawhatchee formation of Florida is also suggested by the presence at Jamesville of the Ostracode *Paracytheridea vandenboldi* and the Foraminifera *Spiroloculina planulata* and *Quinqueloculina seminula*.

[2] Geologist, Ground Water Branch, Water Resources Division, U. S. Geological Survey, Raleigh, N. C.

The Jamesville assemblage, both in number of species and in frequency of occurrence, is skewed sharply towards the warm-water forms. This implies that the thermocline dividing the warm-water forms from the cold-water forms lay considerably to the north in middle Miocene time. This is in contrast with the upper Miocene thermocline, which was to the south, and with the present-day thermocline, considered to be along the Hatteras axis. Further study of taxonomic relationships and specific ranges is necessary before a definite stratigraphic position can be assigned to these deposits on the basis of their microfauna.

A Dome of Brackish Ground Water in the Coastal Plain of North Carolina. H. E. LEGRAND[3] AND J. R. LEGRAND.[4]

A dome of brackish ground water, in the Coastal Plain of North Carolina, between the Cape Fear and Black Rivers, near their confluence, was discovered by the writers during 1953. Its apex, about 2 miles north of Kings Bluff, is marked by an ancient salt pit and by seepage of brackish ground water from surficial sands. This seepage forms a small stream, the chloride content of which is 1,200 parts per million when its flow is at a low stage of about 50 gallons a minute. Several wells less than 100 feet deep yield water too salty to use. At distances of less than 10 miles from the apex of the dome, and completely surrounding it, fresh water is found to a depth of more than 200 feet.

Despite its proximity to two rivers, the area is poorly drained and of low relief. Beneath the surficial sands, which are generally less than 25 feet thick, are Cretaceous deposits including, in descending order, interbedded sands and clays of the Peedee and Black Creek formations and undetermined deposits of earlier Cretaceous age. Regionally the uppermost beds dip gently toward the coast.

Brackish or briny water from the Cretaceous artesian aquifers leaks upward to contaminate the water locally in the surficial sand. The inability of water to leak rapidly through numerous intervening impermeable clay beds, and the great ability of precipitation to dilute shallow brackish ground water when it is replenished rapidly, indicate that only a significant geologic structure could permit upward leakage at a rate great enough to maintain the surficial body or dome of brackish water. Only a fault or an intrusive salt mass is considered capable of breaching the clay beds and allowing the upward movement of an adequate amount of salt water. Until more well data become available the true character of the structure cannot be determined.

Clay Mineralogical Profile from Fresh to Salt Water. MAURICE C. POWERS.

The rivers of the Chesapeake Bay carry in suspension predominantly a well-formed to degraded illite with minor amounts of kaolin and accessory degraded chlorite. Montmorillonoid rarely occurs.

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[4] Industrial geologist, Atlantic Coast Line Railroad, Wilmington, N. C.

Electron microscope photographs of suspended and bottom sediment clays in the James estuary indicate a decrease in floccular size and an increase in crystal size with increasing salinity.

A chloritic clay is forming in the Chesapeake Bay estuaries. Its thermal stability increases with increase in the salinity of the environment and with depth in the sediments. This material seems to arise from the diagenesis of degraded illite in the Chesapeake Bay area, but it has also been observed to result from diagenesis of a montmorillonoid in the Atchafalaya region of the Gulf Coast, and in the Pacific ocean west of San Diego.

The geochemical relations of chloride, sodium, calcium, potassium, and magnesium in the clays of the James estuary support the hypothesis that chlorite is forming in the estuarine and marine environment.

The diagenic state of the chlorite seems to be a function of the concentration of magnesium in the interstitial water.

Clay Minerals and Paleoclimatology. ROY L. INGRAM.

The clay mineral content of a sedimentary rock can be used to infer the climatic conditions that prevailed during the time of deposition of the rock if the clay minerals were derived from mature soils and if the clay minerals have not been altered since deposition. As a result of these limitations, the paleoclimatologic interpretation of clay minerals is limited to non-marine sediments deposited at a slow to moderate rate in a non-stagnant environment. For sediments that meet these qualifications, clay mineral compositions infer certain climates: montmorillonite and illite—dry and hot to cool; illite—wet and cool; kaolin—wet and warm; and gibbsite—wet and hot.

The Adjustments of Land-Derived Clays to the Neuse River Estuary. GEORGE M. GRIFFIN AND ROY L. INGRAM.

Clay fractions from 24 Neuse River Estuary bottom samples were examined by X-ray diffraction to determine their clay-mineral content and to note any diagenetic changes in the clays as they were introduced into a brackish-water environment.

Kaolinite and chlorite were found in all 24 samples, and illite was found in 18 of them. Kaolinite was by far the dominant mineral being introduced into the estuary, but it decreased considerably in amount relative to chlorite and illite as increasingly salty water was encountered downstream. Chlorite increased relatively as kaolinite decreased, and chlorite had become the dominant mineral at the lower end of the estuary. Illite, though very subordinate to both kaolinite and chlorite throughout the estuary, showed a definite increase in abundance in the last few downstream samples and appeared to be increasing more rapidly than chlorite.

The chlorite was thermally unstable and collapsed when heated at 400°C. for one hour; however, it was like normal chlorites in its failure to react with ammonium salts and glycerol.

Geology in its Relation to the Seven Life Zones of Biology and the Seven Language Zones of Ethnology. A. L. PICKENS.

Between the Arctic Ocean and the Gulf of Mexico the language zones of the ethnologist, Eskimauan, Athapascan, Algonquian, Iroquoian, Siouan and Muskhogean strikingly incline to center respectively in the Arctic, Hudsonian, Canadian, Appalachian, Carolinian, and Louisianian of the biologists life zones. We allow a connecting band between the eastern and western Siouans in former times. The Timuquan language zone and the Tropical zone are closely related in the Florida peninsula. Even in modern life, below the still existing Eskimauan and Athapascan zones, forestry, manufacturing, corn, cotton and citrus belts roughly follow the old alignment. A tendency of life, even human life, to arrange itself according to the zones of Thales and others, but even more minutely, is indicated. The Appalachian mountains are the most disrupting influence and bring to the South more northerly plants, animals, and Indians; and astoundingly, Union sympathizers and Republican voters have shown the same tendency in history. Southern tendencies reach farther north along river valleys, both north-flowing and south-flowing, but, especially in spring, earlier phenomena show northern influence in many southern valleys owing to a downflow of cooler air. Some Carolina streams show this markedly when rising in the mountains. The boundary between the Louisianian (Austroriparian) and the Carolinian zones can no longer be thought of as following the noted Fall Line. The two decussate in North Carolina. The more southern zone presses close against the Georgia mountains and apparently invades the coast plain in the Chesapeake area.

Geology of Northeast Angola. E. W. BERRY.

The area of northeast Angola (Latitude 7°20′ to 9°20′ south and Longitude 19°50′ to 10°50′ East) consists of a dissected plateau at an elevation between 700 to 900 meters, sloping north. The Kasai River forms the eastern boundary. Flowing north and to the west are six other northward flowing rivers which join the Kasai in the Belgian Congo. These rivers have many primary and secondary tributaries carrying considerable water even in the dry season. Occasional flats are developed along the rivers and terraces show up at places. Low falls and rapids are fairly common and the drainage is in youth. The divides are broad and in general flat, although there are occasional hills and ridges of older rock which rise above the general surface.

The rocks consist of a basement complex of amphibolite, granites, gneisses, and much metamorphosed schists of Pre-Cambrian age. On this irregular surface are remains of a series of quartzites and phyllites, the Kabara which are Paleozoic in age. In a few places there is the Luana quartzite of indefinite age but probably Paleozoic. These rocks were more or less reduced and some faulting took place. The resulting surfaces have remains of Dwyka Tillite (Karroo) and highly colored varved clays of the Lutoe and in places the red and white deposits (lake beds) of the Cassange with fossil fish and *Esteria*. These were all faulted and a graben developed with a N65° ± E strike and the entire region reduced to a

partial peneplain with the Lutoe and Cassange only preserved in the graben area. It is thought that this surface sloped south, with a relief of about 300 meters. On this irregular surface the sands and gravels (lag gravels) of the Upper Karroo were accumulated, giving us the Lunda series. The faulting about the Cassange and before the Lunda was accompanied by intrusions of Kimberlite. Diamonds are found in the Lunda but not in the Cassange and older rocks. On top of the Lunda and everything else are the Kalahari sands except on some high ridges of the crystalines. On this Kalahari plain the present drainage was developed. This series underwent various silicifications and lateriations as the climate changed and was redistributed several times. It is still being moved, and in some Pleistocene pebble tools are found.

The Plateau is in general covered with heavy coarse elephant grass (8 to 10 feet high) with scattered stunted trees. The river valleys and creeks are usually heavily wooded, while the abrupt slopes are more or less open but covered often with redistributed Kalahari sand so that bed rock exposures are very rare. Most of the detail geology is dug out at the alluvial diamond mines, or from prospecting pits and drill holes.

New Developments in Titanium. EDWARD C. NIXON AND C. EDWARD HOWARD.

The reported mineral compositions of iron-bearing titanium sands are reviewed. The chemical composition of the ore mineral in many of these sands corresponds to that of arizonite rather than ilmenite. X-ray analysis, however, fails to prove that this ore contains the rare mineral arizonite. It is suggested that these sands actually consist of a titaniferous aggregate of intergrown ilmenite, hematite and/or magnetite.

The Amphibians of New Hanover County. JOHN B. FUNDERBURG, JR.

Fourteen species of salamanders and twenty-one species of frogs and toads have been found in New Hanover County. The status and distribution of these forms is given and extensions of range in North Carolina are reported for *Ambystoma opacum*, *Manculus quadridigitatus*, *Pseudotriton ruber ruber*, *Plethodon cinereus cinereus*, *Scaphiopus holbrookii holbrookii*, *Pseudacris nigrita nigrita*, *Pseudacris ornata*, *Hyla crucifer* and *Hyla gratiosa*.

The Population Structure of the Spiders of the Abstract Broomsedge Community in the Piedmont of Southeastern United States. ROBERT D. AND BETTY MARTIN BARNES.

The spider population of twenty-nine stands of the broomsedge community, *Andropogon*, were sampled during June, July and August of 1953. Only quantitative samples were taken and these were restricted to the herbaceous level of the community. All fields studied were located in the Piedmont of southeastern United States, sixteen being in North Carolina, six in South Carolina, five in Georgia and two in Alabama. A total of 5,416 spiders was collected, including 90 species. From the samples collected it was found that there was a very high degree of constancy in the spider population from one field to another, not only

in the species present, but also in their relative densities. It was therefore evident that the abstract broomsedge community (considering only the vegetation) could be said to support an abstract spider population which is characterized by five major species: *Phidippus clarus*, *Mangora gibberosa*, *Eustala anastera*, *Oxyopes salticus*, *Hyctia pikei* and *Clubiona catawba*. The variation present between stands was difficult to correlate with any one factor. A small proportion of these differences could be explained by seasonal changes observed from monthly samples taken in one stand located in Durham Co., N. C. An attempt was made to correlate population differences between stands with the degree of compactness of the broomsedge dominant, other vegetation in the field, age of stand and geographical location. However, no apparent correlation could be detected with any one factor. It is probable that these factors are important but are operating as a tightly knit complex, the component effects of which are difficult if not impossible to separate.

PROCEEDINGS OF THE ELISHA MITCHELL SCIENTIFIC SOCIETY
1953–1954

506TH MEETING, OCTOBER 13, 1953

VIRGIL I. MANN (Geology): *The Relation of Oxidation to the Origin of Iron Ores of Michigan.*

The processes which affected the development of soft iron ores in the Marquette and Gogebic ranges were examined and evaluated by petrographic and chemical techniques. By tracing oxidation through mineral transitions from original rock to ore the conclusion was reached that soft iron ores of these two ranges were the result of the action of mildly alkaline hydrothermal solutions upon silicated iron formations for a long period of time.

Oxidation, as exhibited by major and minor minerals of the iron formation, was the initial step in the development of soft ores. Alterations and oxidation of other rocks affected by the ore forming solutions confirm the processes recognized in the iron formation. Some of the reactions that took place during oxidation were evaluated according to the most recent thermodynamic data.

The steps in soft ore origin on these two ranges are commonly multiple and overlapping; however in their most simple form they are: 1) silication of the original iron formation, 2) oxidation of silicate formation, and 3) leaching of silica from the oxidized silicate rock. The last two steps were brought about by hydrothermal solutions.

VICTOR A. GREULACH (Botany): *Recent Work on Maleic Hydrazide as a Plant Growth Inhibitor.*

Although most of the numerous papers on the effects of maleic hydrazide (MH) on plant growth which have appeared since 1949, when the plant growth-

inhibiting properties of MH were first announced, have dealt with possible practical applications, a considerable amount of information has been secured on the mode of MH action. It is now well established that MH inhibits cell division. The effects of MH on cell enlargement are varied, the cells in treated plants often being larger than those in the controls. Greulach and Haesloop have found that MH inhibits cell enlargement in the pith of young tomato stems, but that cortical cells are two to three times as long in the treated plants as in the controls. The amount of xylem and lignified interfascicular tissue is greatly reduced, due to inhibition of secondary meristematic activity. The inhibition of cell division by MH can account for essentially all of the inhibition of internode elongation in tomato stems, as well as for inhibition of apical growth and lateral stem growth. This casts doubt upon growth inhibition by MH through anti-auxin activity. MH may also have an effect on cell differentiation, as indicated by some of its formative effects and by the fact that Greulach found guard cells halted in various stages of ontogeny in treated bean and sunflower leaves as well as greatly reduced numbers of stomates per unit area.

Various secondary physiological effects of MH include accumulation of carbohydrates, as shown by various investigators, anthocyanin formation, and, as shown by Petersen and Naylor, lower protein content and increased non-protein nitrogen compounds, particularly leucine. MH increases osmotic pressure of sunflower and bean leaf cells, as shown by Greulach, and reduces the rate of transpiration. T'so and Steinberger report that MH reduces auxin-induced water absorption. Greulach has reported that while starch disappears from leaves of treated intact plants more slowly in the dark than from the controls, it disappears much more rapidly from detached treated leaves than detached control leaves.

Primary physiological or biochemical effects which have been reported include anti-auxin activity (Leopold and Klein and other workers), inhibition of respiration (Naylor and Davis and other workers), and decreased membrane permeability (Johnson). On the basis of preliminary data or theoretical considerations, it seems possible that MH might also cause certain trace elements to be unavailable, interfere with protein synthesis, or interfere with the synthesis of thiamine, nucleic acids, or other pyrimidine-containing compounds.

While isonicotinic hydrazide and other open chain hydrazides have been shown to inhibit plant growth, their effects are not similar to those of MH. Since maleimide has been shown to inhibit mitosis in chick fibroblasts it should be worth while investigating its effects on plant growth. MH is actually not a true hydrazide but a pyridazine derivative, 1,2-dihydropyridazine-3,6-dione, so its closest analogues are to be looked for among the diazine group of heterocyclic ring compounds, particularly other pyridazine derivatives. Of the few compounds of this type which have been investigated as regards their effects on plant growth most do not inhibit plant growth. The few which do are much less effective than MH.

Since MH does not inhibit growth or cell division in animals, and seems to have no effects on at least several species of bacteria, fungi, and algae it apparently exerts its effects on phases of metabolism unique to the higher plants.

507TH MEETING, NOVEMBER 10, 1953

A. ROE (Chemistry): *Isotope Effect Studies with C_{14}.*

J. E. WILSON AND T. B. MOORE (Biological Chemistry and Nutrition): *Is Histidine an Essential Nutrient for the Adult Rat?*

Dietary histidine is not required for the maintenance of nitrogen equilibrium in adult humans, but, with one exception, reports in the literature agree that it is required by the common laboratory animals that have been studied in this respect. Histidine is necessary for the growth of the rat, but there has been some disagreement concerning whether or not adult rats require it in the diet. It seemed possible that studies that had indicated a definite dietary requirement for histidine may have been affected by a lack of "non-essential" amino acids in the diets used, by a lack of unidentified nutritional factors, by a failure to maintain adequate caloric intakes in the experimental animals or by a failure to maintain adequate intakes of amino acid nitrogen.

Complete diets were prepared containing 17 amino acids, all known growth factors, and a liver powder to provide as yet unknown dietary factors. This diet supported nitrogen equilibrium in adult albino rats when it was supplemented with histidine. Without added histidine there was a consistent loss of nitrogen by the experimental animals, even when forced feeding was used to maintain caloric and nitrogen consumptions at normal levels or higher. It was concluded that the adult rat is unable to synthesize histidine at a rate sufficient to meet its needs.

508TH MEETING, DECEMBER 8, 1953

GEORGE D. PENICK (Pathology): *Experimentally produced cold-injury in dogs.*

The actual role of vascular thrombosis in the production of frostbite necrosis is debated. Controversial results have been obtained in humans and experimental animals subjected to cold-injury and treated with anticoagulants. To avoid the fluctuations of artificially produced hypocoagulability, twenty local cold injury lesions were produced in hemophilic dogs and compared with similarly produced lesions in normal dogs. Gross and microscopic observations suggested similar courses in the two groups of animals except for the relative failure of edema development in the earlier phases of the hemophilic experiments. This discrepancy was further explored by the determination of the effect of local freezing of tissues on various circulating blood coagulation factors. The antihemophilic factor was found to be depressed about 40% during the first 24–48-hour period after freezing. Circulating platelets were diminished after the freezing of normal dogs but exhibited only a retarded drop in the hemophilic animals. These data suggest that local or systemic clotting occurs in normal animals subjected to cold injury but not in similarly treated hemophilic dogs. They further suggest that a thrombotic role may aid in the overall production of frostbite necrosis but a retardation of development of thrombosis in the hypocoagulable state may result in an early difference of tissue response.

W. A. BOWERS (Physics): *Two New Kinds of Magnetism.*

Recent research has shown the existence of two new types of magnetic behavior, which have been given the names "antiferromagnetism" and "ferrimagnetism" respectively. Antiferromagnetic materials are characterized by a susceptibility which obeys, above a certain characteristic temperature, a Curie-Weiss type of law, but with a negative Curie temperature. Ferrimagnetic materials exhibit permanent magnetization below a characteristic Curie temperature, but their saturation moments are considerably less than one would expect from the known moments of the metal ions of which they are composed. Néel has shown that both these types of behavior can be explained by assuming antiferromagnetic coupling between neighboring ions, i.e. interactions tending to cause antiparallel alignment of the atomic spins; this is just the contrary of the usual situation in ferromagnetic materials. The result is two interpenetrating sublattices with oppositely oriented magnetizations; if the two opposite moments are equal in size, one has antiferromagnetism, if not one has ferrimagnetism. These ideas have received striking confirmation from neutron diffraction studies; since neutrons interact magnetically with atoms, their diffraction patterns show directly the existence of Néel's magnetic sublattices.

509TH MEETING, JANUARY 12, 1954

H. E. LEHMAN (Zoology): *An Experimental Analysis of Embryonic Pigment Patterns in Salamanders.* (This paper appears in full elsewhere in this issue of the Journal.)

W. A. GIBSON (Psychology): *Latent Profile Analysis: A New Multivariate Model.*

It can be shown that the product-moment, about the origin, of a set of n_q points having a zero product-moment about their centroid, is equal to the product-moment, about the origin, of n_q points placed at that centroid. This principle generalizes to triple and higher-order product-moments. Hence it is conceivable that the correlation matrix and the higher-order product-moment matrices could be accounted for by a limited number of mutually exclusive subsamples, each being homogenous enough, with respect to whatever underlying variables are involved, so that all product-moments of all orders within it are zero. The equations which express this are:

$$1 = \frac{\sum_{i=1}^{N} 1}{N} = \frac{\sum_{q=1}^{m} n_q}{N},$$

$$0 = \frac{\sum_{i=1}^{N} Z_{ij}}{N} = \frac{\sum_{q=1}^{m} n_q Z_{qj}}{N},$$

$$r_{jk} = \frac{\sum_{i=1}^{N} Z_{ij} Z_{ik}}{N} = \frac{\sum_{q=1}^{m} n_q Z_{qj} Z_{qk}}{N},$$

$$r_{jkl} = \frac{\sum_{i=1}^{N} Z_{ij} Z_{ik} Z_{il}}{N} = \frac{\sum_{q=1}^{m} n_q Z_{qj} Z_{qk} Z_{ql}}{N},$$

etc., where, for example, Z_{ij} is the standard score of individual i on test j, n_q is the number of people in subsample q, and Z_{qj} is the average standard score of sub-sample q on test j.

Because of a complete identity of form, these equations can be subjected to any method of solution for Lazarsfeld's latent structure equations to yield a set of latent parameters which is, in general, unique for a given battery of tests.

510TH MEETING, FEBRUARY 9, 1954

W. D. CASH, F. S. SEMENIUK, AND W. H. HARTUNG (Pharmacy): *Reproducible Palladium Hydrogenation Catalysts.*

C. E. JENNER (Zoology): *Photoperiodism in Marine Animals.*

511TH MEETING, MARCH 9, 1954

W. J. CROMARTIE (Bacteriology): *The Relationship of Streptococci to Heart and Kidney Disease.*

W. H. WHEELER (Geology): *Cope and Marsh and the Uintotheres—A Study in Taxonomy and Personalities.*

512TH MEETING, APRIL 13, 1954

KERRO KNOX (Chemistry): *Technetium.*

Element 43 is now produced in gram quantities from fission products at Oak Ridge. Studies on this amount of this new element by Boyd and his co-workers confirm in general the previous tracer experiments and show the strong similarity in chemical properties between technetium and rhenium to be expected from their positions in the periodic table.

Preliminary experiments on two further aspects of technetium chemistry both indicate differences in behavior from rhenium. First, a new technique for the preparation of transition metal chlorides was developed to overcome the previous difficulties in the preparation of technetium chloride. Technetium heptoxide and an excess of carbon tetrachloride were sealed inside a glass reaction vessel which was then placed inside a steel bomb containing the amount of water calculated to balance the pressure of the carbon tetrachloride at the reaction temperature. The whole apparatus was heated to 400° C. for several hours. Technetium produced a red compound tentatively identified as the trichloride. Rhenium under similar conditions gives the pentachloride, as other transition elements give their highest known chlorides. Thus, the highest stable chloride of technetium is perhaps the trichloride.

Second, the experiment by Lundell and Knowles (Jour. Research Nat. Bur. Standards **18:** 629, 1937) on the behavior of perrhenate in a Jones reductor was

tried on technetium after it had been repeated successfully with rhenium. Rather than going to the uninegative state and coming out the bottom of the column as rhenium does, the pertechnetate is reduced to some insoluble compound, either the dioxide or more probably the metal, which sticks to and darkens the zinc particles at the top of the column.

513TH MEETING, MAY 11, 1954

CLAUDE L. YARBRO, JR. (Biological Chemistry): *Investigation of Metabolic Role of Acetal Phosphatide.*

At the beginning of the meeting, Dr. Andrews presented a certificate and a check for $50.00 to Mr. Claude Lee Yarbro, as winner of the William Chambers Coker Award in Science for the year 1953–4.

The treasurer's report was read, and The Auditing Committee (M. A. Hill, W. A. Bowers) reported the books in order.

The Nominating Committee (C. S. Jones, W. R. Straughn, and S. Y. Tyree, Chairman) presented the following slate of nominees for the year 1954–1955:
President—W. C. George
Vice-President—W. R. Mann
Secretary-Treasurer—J. C. Morrow

The slate was unanimously elected.

J. C. MORROW, *Sec.-Treas.*

THE LIFE HISTORY OF THE NORTHERN GREENSIDE DARTER, *ETHEOSTOMA BLENNIOIDES BLENNIOIDES* RAFINESQUE[1]

BY WILLIAM E. FAHY[2]

University of North Carolina Institute of Fisheries Research, Morehead City, North Carolina

INTRODUCTION

The Percidae embrace three subfamilies, Percinae, Luciopercinae, and Etheostomatinae. The Etheostomatinae, darters, comprise about one hundred North American species. It is the purpose of this paper to make known the life history of the northern greenside darter, *Etheostoma b. blennioides* Rafinesque.

In the nineteenth and early twentieth centuries the Etheostomatinae were studied from a taxonomic standpoint in which description and distribution were the principal problems. Some investigations treated different aspects of the life

[1] Submitted to the faculty of the Graduate School of the University of Rochester in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Publication of this paper is made possible by a Grant-in-Aid of Research from the *Society of the Sigma Xi-Resa* research fund.

history of darters, such as breeding habits (Reeves, 1907; Seal, 1892), behavior (Jordan and Copeland, 1876*b*), food (Forbes, 1880), and variation (Moenkhaus, 1898). During the second decade of the twentieth century, observations of breeding habits were made by Jaffa (1917) and Reighard (1913), and later by Adams and Hankinson (1928), Atz (1940), Hankinson (1932), Lake (1936), J. Petravicz (1936), W. Petravicz (1938), and Raney and Lachner (1939). Modern methods of fishery biology and ichythyology have been applied to studies of darters as shown in problems of variation (Gosline, 1947), speciation (F. Stone, 1947), and populations (Gerking, 1949).

There is a paucity of information on the life history of the greenside darter. Since Rafinesque's (1819: 419) original description, subsequent publications have treated primarily its taxonomy, description, and distribution (Cope, 1868: 215; Jordan and Evermann, 1896*b:* 1052–53). Jordan and Copeland (1876*b:* 339–40) gave a semi-popular account of habits as observed in an aquarium, and Forbes and Richardson (1920: 292–94, col. pl.) gave additional observations on behavior. *Etheostoma blennioides* was mentioned coincidentally with investigations of other fishes (Hankinson, 1932: 418–19) in which it was noted near the spawning site of cyprinids. Thompson and Hunt (1930) indicated the relative abundance of the greenside darter and Lake (1936: 817) and Raney (1941: 3) described associations of fishes that included *E. blennioides.*

This study commenced in the fall of 1946 and continued through four years. The field studies were carried on chiefly in Salmon Creek, Lake Ontario drainage, Monroe County, New York.

Gerking (1949: 301, 304) and Lachner *et al.* (1950: 92–111) have contributed studies of *blennioides* during the completion of mine. Gerking collected greenside darters from sections of different Indiana streams and computed the average percentage weight contributed by the species to the total weight of different segments of the fish population. Lachner and his colleagues completed studies on age and growth, sex ratio, and habitat; their material was more limited than mine, and it was collected from a large river in the Upper Ohio drainage basin.

SYNONYMY

Etheostoma blennioides Rafinesque. Greenside Darter

Etheostoma blennioides. Rafinesque, 1819: 419 (original description); 1820*a:* 169, and 1820*b:* 37–38 (description; erected subgenus *Diplesion*). Kirtland, 1840: 348, Pl. VI, Fig. 1 (characters; misidentification, figured another species). Agassiz, 1854: 304–05 (misidentification). Vaillant, 1873: 102 (misidentification). Opinion 14 International Commission Zoological Nomenclature, 1910: 25–27 (type of genus). Jordan, Evermann and Clark, 1930: 286 (synonymy in part).

Diplesium blennioides. Agassiz, 1846: 332 (*-ium* the proper grammatical ending). Jordan and Copeland, 1876*a:* 133–36 (synonymy). Jordan, 1877*a:* 21–23 (synonymy; *Etheostoma flabellaris* Raf., the type of *Etheostoma* Raf.); 1877*b:* 15 (synonymy).

Diplesion blennioides. Girard, 1859: 101 (refused to recognize as type of *Etheostoma* Raf.). Jordan and Evermann, 1896*a:* 361 (synonymy in part).

Hyostoma blennioides. Jordan, 1876: 93 (synonymy).

Hyostoma newmanii. Agassiz, 1854: 305 (description). Vaillant, 1873: 102 (*species inquirendae*).

Diplesium newmani. Jordan, 1877*b*: 15 (synonymy; considered *blennioides* Raf., *newmani* (Agassiz), and *simoterum* (Cope) the three species of *Diplesium*).

Pileoma cymatogramma. Abbott, 1860*a*: 327 (description).

Percina cymatogramma. Abbott, 1860*b*: 479 (*Percina* Haldeman congeneric with, and prior to, *Pileoma* DeKay).

Hyostoma cymatogrammum. Cope, 1865: 82.

Hyostoma blennioperca. Cope, 1868: 214–15 (description; generic characters); 1870: 270 (synonym of *H. cymatogrammum* Abbott). Vaillant, 1873: 102 (synonymy).

DESCRIPTION

Specific characters.—Satisfactory descriptions are given by Jordan and Evermann (1896*b*: 1053) and Forbes and Richardson (1920: 292–93). The singular character for the identification of this species is the pronouncedly inferior mouth, a condition exaggerated by the overhanging gibbous snout (Hubbs and Lagler, 1947: 84, fig. 45) in which the premaxillaries are fused to the preorbitals at the sides. This peculiar condition of the snout and mouth is characteristic of both sexes in all age-groups, for, in the youngest specimens taken by me (young-of-the-year, August 8, 1948) it was as evident as in older fish.

Color notes and pattern.—The greenside darter exhibits the usual coloration condition of the subfamily in having the adult males more intensely colored than adult females or juveniles. The most satisfactory color description is that of Forbes and Richardson (1920: 292, col. pl.). With certain modifications that description is applicable to an adult male of the Salmon Creek population during the winter months. In the population studied, eight, green, Y-shaped, lateral blotches or bars are usually present, occasionally seven. In the male, these bars extend over the dorsum to present the appearance of bands; in the female, the branches of the Y's meet to form a series of arches along the side (Jordan and Gilbert, 1882: 497; Jordan and Evermann, 1896*b*: 1053; Evermann and Clark, 1920: 441; Forbes and Richardson, 1920: 292). Ventrally, the tapering bases of the Y's below the lateral line extend almost to the mid-ventral line. Pelvic and anal fins are more fleshy in older fish than in younger ones, and more fleshy in males than in females. Gold flecks located at intervals along fin rays appear as three or four concentric arcs when a fin is viewed in its entirety; in less fleshy fins the flecks are more apparent.

The following color notes are based upon living specimens in the stream. At the completion of spawning activity the brilliant coloration of the adults disappears within a period of a few days. This loss can be observed as early as the first week of June, and by the third week all specimens show the relatively drab summer coloration. Summer coloration persists until September. At this time, especially in males, the greens become slightly darker; later in October and November when water temperatures fall to a range between 50° and 40°F. a

noticeable increase in the intensity of coloration occurs. By late March and early April the breeding colors can be recognized.

Jordan and Gilbert (*loc. cit.*), Jordan and Evermann (*loc. cit.*) and Evermann and Clark (*loc. cit.*) stated that the young are similar to adult females. This similarity is limited to the summer and autumn period, because juveniles begin to assume their respective sexual coloration and pattern during January and February.

A peculiar color phenomenon has been observed in five large males. An extremely dark green (appearing black) extends over the entire caudal peduncle and caudal fin areas. All such males were in or had passed through the third summer. This coloration did not appear to be related with any particular season or activity, because specimens were taken as follows: one in April, 1947; two in May, 1948; one in August, 1948; and one in September, 1950. One male collected in May, 1948 was brought into the laboratory to spawn with a female of comparable age. Within a week it had lost the black tail coloration and appeared like any other male of similar age. This male spawned when the black tail was in evidence and after it had disappeared. No explanation for this color phenomenon is offered.

Adult male during breeding season. Fish spawning for third or fourth season.—Head dark green above; ground color of the body dark olive-green above lateral line, somewhat lighter below; venter olive-green; region of isthmus and throat suffused with dark green; Y-shaped bars dark green; small orange-rufous spots of the side distributed mostly above the lateral line in the first four longitudinal scale rows, sometimes appearing as irregular horizontal lines. Dorsal fins nearly transparent. First dorsal with vivid orange-rufous band at base extending about one-third the height of the fin but restricted to the membrane between fin rays; immediately above, a band of grass-green separated from an outer band of blue-green by a clear area, relative heights of the different bands varying, but clear area usually the smallest, a thin edging of dark green around entire fin; gold flecks of fin rays apparent. Orange-rufous band at base of second dorsal not so vivid or as high as in first; immediately above, a deep blue-green bordered with a translucent white; gold flecks more conspicuous than on first dorsal. Caudal fin opaque; suffused with dark green. Anal fin opaque; deep blue-green from base outward to ventral and posterior opaque white border. Pelvics deep blue-green from base outward to opaque white border anteriorly and ventrally. Pectorals transparent; grass-green to blue-green with conspicuous gold flecks; anterior and ventral borders often with thin white edging.

Males spawning for first or second season.—Head dark green above; ground color of body somewhat lighter than in older males; venter creamy white to light green; region of isthmus and throat lighter green; Y-shaped blotches dark green; orange-rufous spots of the side as in older males. Dorsal fins as in older males. Caudal fin translucent blue-green; edged dorsally and posteriorly with dark green; gold flecks arranged more or less in vertical lines. Other fins colored as in older males.

Adult males, post-spawning season.—Head green above; ground color of body

light olivaceous above lateral line, somewhat lighter below; venter creamy white; region of isthmus and throat grayish; Y-shaped blotches grass-green; orange-rufous spots not as conspicuous as during breeding season. All fins transparent. Orange-rufous band of the first dorsal less vivid and not as high as during spawning; distal area of fin grass-green; no edging present; gold flecks conspicuous. Orange-rufous band of second dorsal reduced to a series of spots along fin base; central area of fin grass-green, sometimes blue-green; no white border; gold flecks prominent. Caudal fin grass-green; no edging present; gold flecks prominent. Anal fin grass-green and white border reduced; gold flecks prominent. Pelvic fins grass-green to blue-green in the distal area; white border present anteriorly and ventrally; gold flecks conspicuous. Pectorals with some light green; gold flecks prominent.

Adult female during breeding season.—Head lighter green above than in male; ground color of the body olivaceous above the lateral line, somewhat lighter below; venter creamy white with netlike strands of light green; region of isthmus and throat like venter; the branches of the successive Y-shaped blotches meeting at the level of the lateral line and forming a continuous series of dark green arches, a dark green spot beneath each arch; orange-rufous spots of the side conspicuous. Fins of female less colorful than male. Orange-rufous band at base of first dorsal fin apparent between fin rays, not as vivid or as high as in male; remainder of fin as in male but grass-green in colored areas; no edging present; gold flecks more conspicuous than in male. No orange-rufous basal band in second dorsal; entire fin grass-green, sometimes blue-green in older females; gold flecks conspicuous. Caudal grass-green to blue-green throughout; gold flecks arranged in vertical lines. Anal and pelvics similar to those of male; white borders reduced; gold flecks prominent. Pectorals as in male.

Adult female, post-spawning season.—Head yellow-green above; ground color of body yellow-green to brown above the lateral line, lighter below; venter creamy white; region of isthmus and throat like venter; Y-shaped pattern of sides, and spot beneath the arches, grass-green; orange-rufous spots of sides brownish. All fins transparent, gold flecks prominent. Orange-rufous basal band of first dorsal brownish, about one-half the height during breeding season. In older females the first and second dorsal light green, colorless in younger ones. Anal light green, white edging present. Pelvics light green. Pectorals colorless.

Juvenile.—Coloration and pattern similar to that of adult female (Jordan and Gilbert, 1882: 497). This similarity apparent only during summer and autumn. Fins of juveniles more lightly colored than those of adult female. Fleshiness of anal and pelvics only suggested. Orange-rufous band of first dorsal present in both sexes, but not as high as in adult female. Orange-rufous spots of the sides not as numerous or as conspicuous as in adults.

DISTRIBUTION

General.—Jordan, Evermann and Clark (1930: 286) gave the range of *blennioides* as extending from New York west to South Dakota and Kansas, thence southward to the lower Alabama Basin. Hubbs and Lagler (1939: 31) first used

the trinomial, *blennioides*, recognizing a subspecies in the Great Lakes drainage, and a southern form *newmanii* (Kuhne, 1939: 93, fig. 59). The southern limit of the species is not known. Raney (1941: 3) collected *E. b. blennioides* from the New River system, Ashe and Watauga counties, North Carolina. In the west, Bailey (1951: 189) removed *E. b. blennioides* from the faunal list of Iowa. New York state surveys along the southern shore of Lake Ontario show this darter in streams of Niagara, Orleans, and Monroe counties (Greeley, 1940: 79), and the tributaries of the Clyde River in Wayne and Seneca counties (Greeley, 1928: 104–5).

Local. Character of the stream.—Salmon Creek rises in the marsh lands of the extreme eastern border of Orleans county, flows in a general northeasterly direction to empty into Braddock's Bay, an arm of Lake Ontario. The stream extends for a distance of 23 stream miles by map measure. From its source to the point where the Barge Canal crosses over it the stream varies in width in the summer from 2 to 7 feet and in depth from a few inches to one foot. Downstream from the Barge Canal to the town of Hilton the width varies from 15 to 25 feet and the depth from 6 inches to 3 feet. Below Hilton stream flow is slower and depth increases as the stream approaches Braddock's Bay.

From the source to the Barge Canal crossover the stream bottom is composed of rubble with short stretches of sand and gravel; downstream from the Barge Canal to Hilton the bottom composition is rubble and gravel with many flat rocks and boulders in the riffle sections, sand or gravel in the quiet stretches, and some bedrock areas in both riffles and quiet reaches. Below Hilton the bottom is sand and silt with occasional boulders. All greenside darters from this stream were collected or observed between the Barge Canal crossover and the confluence of Tributary 2 (Brockport Creek), a distance of approximately 9 stream miles. The bulk of the darter population of Salmon Creek is probably contained between these two points because above the Barge Canal the stream often becomes dry during July and August, and below the confluence of Tributary 2 the water is polluted with cannery wastes carried by the tributary.

In addition to drainage and runoff water from the area a considerable amount of water is supplied to Salmon Creek by the Barge Canal through overflow valves at the point at which the canal crosses over the stream. The stream carries its greatest volume of water in March and April because of early spring rains and runoff from late snows. There is little widening of the stream at this time, but it is considerably deepened. Riffles 6 inches deep in summer are 2 or more feet deep at this time, and pools increase from a summer depth of 2 or 3 feet to nearly 5 feet. The water is clear, white, and with a slow to moderate current in summer; in early spring it is roily with swift to torrential current. Temperature records over a period of four years show a minimum water temperature of 33°F. for flowing water and a maximum of 83°F. (Fig. 4). Winter observations from 1947 through 1951 show that the stream did not freeze over completely; however, in these years there were no prolonged periods of extreme low air temperatures. The water temperature fluctuates as much as 6°F. per day during the summer.

Habitat.—Forbes and Richardson (1920: 293–94), Hubbs and Lagler (1947:

89), and Lachner *et al.* (1950: 93) state that the greenside darter is a fish of the riffles where there is a growth of aquatic vegetation. This condition exists in Salmon Creek. Lachner *et al.* (1950: 94) state that *blennioides* avoids the water above the riffle, that is, the quiet water areas. Salmon Creek studies indicate certain departures from strictly riffle habitation depending upon sex, age, and season of the year.

At any time of the year, all age-groups of both sexes are found in the riffles, and in deeper riffles greenside darters are more abundant than in shallow ones 2 or 3 inches deep. During the winter and colder months of the fall when water temperatures are 45°F. and lower, juveniles and adult females of age-group I (Hubbs, 1943: 260) are frequently found in quiet water. When in quiet water these fish do not appear to frequent any one ecological situation more than another. They are observed in midstream over bedrock or gravel bottom, completely exposed, and also along the protecting edge of stones or growths of water cress, *Nasturtium officinale*. The number of juveniles and adult females found in quiet water is small compared to that found in riffles during the same period. Possibly these fish are only moving from one riffle to another, but it is interesting that no older females or adult males have been taken in similar situations. This cold month distribution of juveniles and age-group I females as found in Salmon Creek may not apply to other streams, especially the larger ones. However, in future studies concerning the problems of sex ratio, populations, and ecological associations such a cold month distribution should be considered as possible.

There are more adult males in deeper riffle sections than in shallower ones, and within any riffle there is evidence of segregation of males according to size. In the shallow riffles, about 4 inches deep, large adult males (68.0 mm. S. L. or larger) are seldom found. When found there, such males are invariably taken from beneath the largest alga-covered rocks or boulders in the swiftest part of the riffle. No smaller male was ever collected from beneath a rock when a larger male was present. In deeper riffles the proportion of large to small males increases. Here again the largest males are found under the edges of the largest alga-covered rocks or boulders, to the exclusion of smaller males, but very frequently the large males are taken in the open water of the deeper riffles together with smaller males. Large males can consistently be captured in a few places in Salmon Creek where the bedrock bottom seeks a lower level abruptly over a stepwise series of ledges. At the bottom of many of these ledges shallow recesses occur beneath the overlying rock. In these spaces the largest males can be found during the summer. Large females and juveniles of both sexes are frequently found in the same places.

The large females (age-groups III and IV) are found as often in the shallower riffles as in deeper ones. Larger females are very often collected with large males beneath the largest rocks and boulders in the riffles, and this association is apparent throughout the year. The younger, smaller darters do not appear to segregate according to sex or size. It should be noted that smaller females are often found under the large rocks with the large males and even when large females are present.

The natural habitat of newly-hatched larvae is not known. The use of various

combinations of mesh sizes and net types produced only negative results although many different ecological situations were searched after three different spawning seasons. Larvae of many associated cyprinids and the centrarchid, *Ambloplites r. rupestris*, were easily collected. That the methods used were faulty is indicated by the fact that no larvae of *Etheostoma f. flabellare* was taken, yet this darter spawns within the spawning period of *blennioides* and is more abundant.

The newly-hatched larvae of *blennioides* apparently do not remain at the site of hatching, between, or upon, the filaments of *Cladophora*. Several hundred alga-bearing rocks were searched during three successive spawning seasons but no larvae were found. The possibility exists that these larvae are swept downstream to become lodged under protecting pebbles and stones, but search of such areas, including the sifting of sand and gravel, failed to show any. The earliest juveniles were taken in shallow riffles (August 8, 1948).

Very little work was done to determine whether the distribution of *blennioides* differs between night and day. On two occasions, during spring months, attempts were made to observe and collect darters during hours of darkness. These operations indicated that the distribution of darters during the night is no different from that during daylight hours.

Plant associations.—The only aquatic plants in Salmon Creek associated with the greenside darter are the water cress, *Nasturtium officinale*, and the filamentous green alga, *Cladophora* sp. The water cress is concerned only in that juveniles and young adult females are collected along the edges of growths of this plant during cold months. The algal association is closer. Different authors (Forbes and Richardson, 1920: 293–94; Hubbs and Lagler, 1947: 89) have observed that the greenside darter is commonly associated with algae-covered rocks in riffles. Greenside darter eggs were taken only from the filaments of *Cladophora* in Salmon Creek. This alga is restricted to riffles, and in winter it is further restricted to the deepest and swiftest part of the larger riffles.

Two other plants occur in the main study area, the water weed (*Anacharis* sp.) and an emergent pond weed (*Potamogeton* sp.), but *blennioides* was not observed near these.

Animal associations.—The greenside darter is primarily a riffle inhabitant and, in considering the association of other animals with it, it is desirable to divide the riffle section of Salmon Creek into two ecological components; first, a comparatively small area of riffle denoted as the "crest" where the water from upstream seeks a lower level suddenly and "white water" is usually formed; second, the rest of the riffle section above or below the crest area. With such an arbitrary definition it is possible for more than one crest to occur within a given riffle, depending upon its length and the stream bed gradient. Salmon Creek, a relatively small stream, has short riffle sections (up to 200 feet in length) and usually one crest to a section.

There does not appear to be any difference between riffle sections, in the faunal composition, but within any one riffle there are pronounced differences between the crest area and the remainder. Throughout the entire length of a section the fishes most commonly associated with *blennioides* are the barred

fantail darter, *Etheostoma f. flabellare;* the hog sucker, *Hypentelium nigricans;* and the river chub, *Hybopsis micropogon*. These species are collected consistently with *blennioides* in all parts of the riffle, at all times of the year, and these are the only forms taken consistently with *blennioides* at the crest area. The stonecat, *Noturus flavus*, was collected three times and only at the crest.

Associated with the greenside darter in the riffles above or below the crest are *Etheostoma nigrum olmstedi, Percina caprodes semifasciata, Semotilus a. atromaculatus, Hybopsis biguttata, Exoglossum maxillingua, Rhinichthys a. atratulus, Notropis rubellus, N. cornutus chrysocephalus, Pimephales notatus, Campostoma a. anomalum, Micropterus d. dolomieu, Lepomis gibbosus*, and *Ambloplites r. rupestris*. With the exception of *P. caprodes semifasciata, L. gibbosus, A. r. rupestris* and *M. d. dolomieu* all of the above forms are taken on occasion at the crest, but occur too infrequently to be a significant part of its fauna. *E. n. olmstedi, S. a. atromaculatus, H. biguttata, N. rubellus, N. c. chrysocephalus*, and *P. notatus* move back and forth between riffles and quiet water and are consistently collected near the upper and lower limits of a section.

The data in Table 1 enumerate the associates of *blennioides* in this particular stream. If it is assumed that all species within a particular habitat have equal chances of being collected, then the figures of Table 1 indicate the relative abundance of a species if comparison is made between species of the same habitat preference.

A study of the collection dates of the 1784 seine hauls shows a periodicity in that the cyprinids and centrarchids appear less frequently during the colder

TABLE 1

Frequency of occurrence of different fishes in 1784 collections made in all months of the year from October, 1946 through October, 1950 in Salmon Creek, Monroe County, New York

Species	Frequency	% Frequency
Etheostoma f. flabellare	1064	59.6
Etheostoma b. blennioides	692	38.8
Etheostoma nigrum olmstedi	55	3.1
Percina caprodes semifasciata	5	0.3
Hypentelium nigricans	200	11.2
Catostomus c. commersoni	18	1.0
Noturus flavus	3	0.2
Hybopsis micropogon	688	38.6
Hybopsis biguttata	214	12.0
Pimephales notatus	276	15.5
Notropis rubellus	225	12.6
N. cornutus chrysocephalus	409	22.9
Semotilus a. atromaculatus	34	1.9
Exoglossum maxillingua	18	1.0
Rhinichthys a. atratulus	1	0.06
Campostoma a. anomalum	178	10.0
Lepomis gibbosus	11	0.6
Micropterus d. dolomieu	9	0.5
Ambloplites r. rupestris	5	0.3

months, November through March. *Hybopsis micropogon* does not show this however. *Percina c. semifasciata* is collected only during summer months.

Animal associates other than fishes are: the mudpuppy, *Necturus m. maculosus*, quite common under large flat rocks in the riffles; the crayfish *Cambarus bartoni*, generally abundant; the water snake, *Natrix s. sipedon*, very common.

Predators.—No direct evidence was obtained that any animal preyed upon the larval, juvenile or adult stages of the greenside darter. There are a number of animals that might be considered potential predators. These include: four species of fishes, the northern smallmouth bass (*Micropterus d. dolomieu*), the northern rock bass (*Ambloplites r. rupestris*), the yellow pikeperch (*Stizostedion v. vitreum*), and the northern pike (*Esox lucius*); two reptiles, *Natrix s. sipedon*, and the common snapping turtle (*Chelydra s. serpentina*); and three birds, the belted kingfisher (*Ceryle alcyon*), the great blue heron (*Ardea herodias*), and the little green heron (*Butorides virescens*). Of these animals the water snake is the most abundant in Salmon Creek.

The eggs of *blennioides* may suffer heavy depredation from some undetermined predator. From May 2 through May 6, 1950 in three different locations along the main study area, spawned eggs attached to alga-covered rocks were counted by hand, left in place in the stream, and re-counted about 24 hours later. To preclude the possibility of the disappearance of eggs by hatching, only rocks that bore eggs of an early stage (before the pigmented-retina phase) were selected. In one instance, 350 eggs were counted from four rocks; the following day only 160 eggs could be totaled from these rocks, and two rocks were practically stripped of eggs. In the second instance, of 225 eggs on three rocks only 80 could be found a day later, and one of these rocks was almost devoid of eggs. In the third instance, 725 eggs were counted on five rocks and only 390 were found later; two of these rocks had nearly the original count and the loss was distributed evenly among the other three. During this egg-counting period the stream was at a normal water level for May (1 to 2 feet at the riffles), so there was no possibility of the eggs being dislodged by mechanical action of the water. There is no reason to believe that counting the eggs and handling the rocks could have been responsible for the loss, because the eggs are firmly attached to the algal filaments. The eggs were probably removed by one or more predators.

Adult greenside darters may possibly feed upon their eggs. On one occasion in the laboratory two adult females and one adult male ingested 37 eggs which had been spawned by one of the females 72 hours previously. This was the only known instance of such cannibalism, although many hundreds of eggs were spawned in the laboratory and permitted to remain in the same compartment with the parents and other darters for several days. Hankinson (1932: 418–19) mentioned that *blennioides* with other fishes was either spawning or searching for eggs in a riffle. Stomach examinations of both sexes and different age-groups of greenside darters in Salmon Creek during the spawning season showed no eggs of any kind in the contents. Lachner (1950: 229–36) found considerable amounts of *Cladophora* in the stomachs of *Hybopsis micropogon* and *H. biguttata* from a stream only seven miles from Salmon Creek. It is possible that these cyprinids

could be egg eaters, the alga having been ingested during the process. (Only Lachner's collection of June 10, 1940 could have contained greenside eggs because all other collections were made at a time when eggs would have been hatched.) Lake (1936: 817–18) suggested that small crayfish may feed upon the eggs of *E. f. flabellare*.

GENERAL BEHAVIOR

Manner of locomotion and the resting position.—Lacking an air bladder and living in swift water the greenside darter is forced to be a bottom dweller. Most of its life is spent lying on the stream bottom under a protective overhanging edge of rock. Movement is accomplished in a sudden dash for a distance of several inches or feet. The greatest distance covered at one time by an observed darter in the stream was approximately 30 feet; this darter moved in a series of dashes about 5 to 8 feet in length. From observations made in the laboratory troughs and in the stream, it is clear that most of the power for these dashes is supplied by the caudal fin. Forbes and Richardson (1920: 279) stated that the pectoral fins were the agents chiefly responsible for the rapid movements of various darters. The pectorals are primarily steering aids in the greenside darter.

When the greenside darter comes to rest upon the bottom of the stream or the slanting side of a stone, then moves forward a few inches or a fraction of an inch to gain a better vantage point, the forward movement is accomplished by the backward sweep of the pectorals. Possibly such forward movements are aided by the forward thrust produced by the expulsion of water through the gill clefts as described by Breder (1924). During spawning activity, the approach of the male to the side of the female, prior to the spawning embrace, is accomplished with no visible movement of any of the fins. Here it is possible that forward movement is due solely to expulsion of water through the gill slits. In a few instances in the laboratory the greenside has been observed to move backward for a very short distance, usually not more than one or two inches, and during these movements the pectorals are swept forward.

The weight of the body is distributed upon the pelvic and caudal fins and the base of the caudal peduncle when the fish comes to a position of rest. With the weight thus distributed the head is raised several millimeters above the bottom, and the angle of the body axis to the horizontal is approximately ten degrees. When in this resting position, the large prominent eyes of the greenside increase its appearance of alertness. Forbes and Richardson (1920: 294) often observed this darter in the resting position with the pelvics upon a pebble or piece of vegetation. In such a pose the angle of the body to the bottom is increased. Regardless of the degree of this angle, the tail region is invariably held at a sharp angle to the body axis, either to right or left, thus giving rise to the characteristic "snake-like" position described by Forbes and Richardson (*loc. cit.*). This resting position has been observed to be maintained for an hour or more in the laboratory.

Reaction to stimuli.—No experiment was designed to test the reaction of the greenside darter to any stimulus, but certain observations were recorded.

There was no consistent response to current direction. The long axis of the body of both male and female was held at different angles at different times to the current direction in the laboratory. The rate of water flow during these observations was only one-half gallon per minute, compared with several hundred gallons per minute in the stream, and therefore the observed responses may have been atypical. All females faced upstream during a courtship display witnessed in the stream. Attending males assumed a position over them, head upstream, but at a slight angle to current direction. When feeding, either in the laboratory, or in the stream, the fish failed to orient themselves in any consistent manner.

The greenside darter is very sensitive to white light and bright red light. White light of sufficient intensity for observation is too intense for the fish. They immediately seek cover beneath rocks or strands of alga and, even though the light is allowed to remain for more than an hour, the fish will not emerge from the hiding places. When a 10-watt, white, frosted bulb is allowed to shine continuously over the rearing troughs for successive nights, the fish move about freely, but when an observer approaches the windowed aperture they seek cover. Under such light the fish react as in daylight. With a 10-watt, red, frosted bulb the fish react in the same way. However, by using a 10-watt, red, photographic bulb (Mazda) an observer can approach the window without alarming the fish. Even sudden movements by the observer do not then excite the fish.

Forbes and Richardson (1920: 94) observed that sudden movements or jarring of the container send the greenside into a state of violent agitation. Moving food organisms stimulate the fish to attack and eat, but non-active living forms, or dried food preparations, cause no response.

FOOD

Manner of taking.—On four separate occasions the greenside darter was observed during feeding activities in the stream. The subsequent capture of the observed fish showed the food organisms to be dipterous insect larvae, *Simulium*. When feeding upon the side of a rock that was approximately parallel to the direction of stream flow, the fish places the long axis of the body along the rock face, head upstream, underside of the body toward the rock, tail bent in the characteristic manner away from the rock with the posterior margin of the caudal fin in contact with the stream bottom. The downwardly directed tail appears to serve as a temporary holdfast while feeding. Forward motion along the face of the rock and motion to the left or right (up and down the face of the rock) is accomplished by the movement of one or both pectorals. With each forward move a new hold is obtained upon the stream bottom by the caudal fin. On one occasion it required about four minutes for a darter to feed over a distance of seven inches. After leaving this rock the fish retired to shelter. An examination of the rock showed that sixteen *Simulium* larvae remained attached. Perhaps these larvae were too firmly attached to be removed.

When feeding from the accessible underside of a rock, the maneuvers of the darter are more elaborate. To an observer it appears that the fish is trapped on its side beneath the edge of the rock, the head and anterior portion of the body

being hidden from view while the visible part of the body wriggles with intermittent lashings of the caudal fin. In this sidewise position the fish moves backward and forward along the underside of the rock. During this maneuver the entire tail region is bent toward the bottom with the tip of the caudal fin in contact with it. It appears that the tail acts not only as a holdfast, but also as an organ of propulsion, as evidenced by the tail lashings.

After the above feeding activities were witnessed in the stream, field conditions were simulated in the laboratory so that an observer could actually see what was taking place. Although the rate of water flow in the troughs could be maintained at a maximum of only two gallons per minute, the feeding activity was found to be essentially the same as in the wild. Rearing trough observations verify those made in the stream which recognized the pectorals as the principal means of forward and backward movement during feeding. In the laboratory the tail is not lashed when the fish is feeding on the underside of a rock, but perhaps the slower rate of flow does not necessitate such action.

When the undersurface of the rock is raised to a point approximately two inches above the bottom of the trough, feeding fish continue to ingest food from a sidewise position even though there is sufficient space to approach the undersurface of the rock in a different manner. In such a position the pectoral fins are used as stabilizers as well as for forward and backward movement; that is, the fins are used to maintain the head of the fish near enough to the rock to feed.

Enchytraeus worms, fed from a standard surface feeder, are frequently ingested before falling to the bottom of the trough, but most of the wriggling worms are eaten from the bottom. When *Gammarus* or large *Daphnia* are introduced to the troughs, the fish rush savagely after them. *Gammarus* is invariably struck at about mid-body, held crosswise in the jaws, and ingested while the fish lies in the resting position. Inch-long pieces of earthworm, *Lumbricus*, are seized by one end and threshed about by the fish; several minutes are required for the darter to ingest one.

Time of taking.—Stomach examinations were made of specimens of both sexes of the first three age-groups in the first week of each month of 1948. An organism in a stomach was counted as one if half or more of that organism was present. The limitations of this method of stomach analysis are recognized. The frequencies of the organism count, according to sex, growing season, and month are shown in Table 2. Each count represents the total number of organisms in one stomach. May was considered the first month of the growing season because some postlarvae could have begun to feed during this month. In the first growing season the months of May, June, and July are blank because postlarvae or juveniles were never collected in the first three months of life. Because more food organisms were counted in specimens collected in August, this month is arbitrarily taken as the period of maximum feeding and counts for all other months are expressed as a percentage of it.

Feeding occurs in both sexes in every month of the year according to the data in Table 2. There are indicated differences between the sexes and between seasons within each sex. Older fish contain more organisms on the average than the

TABLE 2

Number of food organisms found in the stomachs of 33 male and 33 female Etheostoma b. blennioides *of the first three growing seasons, collected in the first week of each month of 1948 from Salmon Creek, Monroe County, N. Y.*

Month	Males					Females				
	Growing Season			Total	% Freq.	Growing Season			Total	% Freq.
	1	2	3			1	2	3		
May	—	72	108	180	56.3	—	29	22	51	14.5
June	—	39	114	153	47.8	—	34	20	54	15.4
July	—	94	218	312	97.5	—	118	207	325	92.6
Aug.*	14	70	236	320	100.0	12	147	192	351	100.0
Sept	72	56	127	255	79.7	63	69	121	253	72.1
Oct	74	92	47	213	66.6	41	107	72	220	62.7
Nov	0	35	80	115	35.9	18	37	76	131	37.3
Dec	21	32	0	53	16.6	17	0	10	27	7.7
Jan	7	0	37	44	13.8	3	14	0	17	4.8
Feb	1	24	21	46	14.4	0	17	22	39	11.1
Mar	13	0	41	54	16.9	18	31	28	77	22.5
Apr	31	21	112	164	51.3	14	21	27	62	17.7

* Collection made August 8.

younger ones. Of the 66 stomachs only 7 were empty, and these were in specimens collected in the period from November through March.

Feeding activity of the males can be related to the water temperature of the stream (Fig. 4). Males feed least from December through March, when water temperatures are lowest. The amount of feeding triples with rising water temperature in late March and early April although the organism counts indicate that this increased feeding is only approximately one-half the amount of August. This amount of early April feeding continues through May and June; possibly spawning activity in this period causes this amount of feeding to be maintained, rather than to be increased as might be expected from the rising water temperature for that period. Post-spawning feeding activity in July is almost as great as the maximum of August, when water temperatures are highest. Water temperature starts to decrease in the last days of August, and the September stomach examinations reflect this by showing only 80 percent of the number of organisms found in August. With continued decrease in water temperatures in October and November the frequency of organisms in stomachs falls to 67 and 36 percent, respectively.

Female feeding activity is lowest in the winter months. With the slightly warmer water temperatures of early March, the activity increases to approximately 23 percent of the August maximum. The spawning season, April-May-June, may influence feeding activity of females more than males (Table 2). It is realized that only a few specimens are involved in this study, but subsequent stomach examinations of breeding females support these data. In the July sample, three weeks after the close of the spawning season, feeding activity increased about sixfold to 93 percent of the August maximum. The subsequent feeding ac-

tivity through summer and autumn is comparable to that of the males for the same period.

Observations in the laboratory show that captive specimens feed at all hours. The stomach contents of Table 2 represent fish collected during the late morning or early afternoon and presumably the food organisms were ingested in daylight hours. Other stomach examinations performed on fish collected in the first hour of daylight showed organisms that presumably were ingested during hours of darkness. On two occasions of night collecting (spring), food organisms occurred in all 22 stomachs. Observations made on wild feeding fish were made during daytime.

Type of food taken.—Postlarvae, never taken from the stream, survive in the laboratory for at least ten days following yolk sac absorption, on a diet of plankton from the stream. Juveniles in August have large *Daphnia* and *Simulium* larvae in the stomachs. Older juveniles and adults feed almost exclusively upon insect larvae. Occasionally in the colder months, juveniles and females of two growing seasons feed upon *Gammarus* in addition to insect larvae. Forbes (1880: 702–03), working with 15 species of darters, but not the greenside, found that insect larvae comprised the greater part of the diet of each darter.

Six orders are represented among the insects ingested by *blennioides* in Salmon Creek. The orders are as follows according to frequency of occurrence: Diptera, Tricoptera, Ephemeroptera, Plecoptera, Coleoptera, and Odonata. The dipterous genera, *Simulium* and *Chironomus* with the tricopterous genera, *Hydropsyche* and *Rhyacophila*, make up the great majority of food organisms. The only other important order is the Ephemerida, represented in the winter months by *Iron* and *Heptagenia*. The other three orders are represented occasionally by the stonefly, *Perla;* the waterpenny, *Psephenus;* and the burrowing dragonfly, *Gomphus*.

All these insects, with the exception of *Gomphus*, are of the type found clinging to the different surfaces of rocks in the riffles. Tests were made repeatedly in which rocks of a given riffle section were examined for food organisms and then the stomach contents of greensides captured subsequently from the same riffle were examined. Consistently the same kinds of organisms as those found on the rocks are found in the stomachs. Such examinations suggest that the greenside darter obtains its food according to availability, as McAtee (1932: 144) concluded for birds, and Hamilton (1940: 355–56) concluded for the newt, *Notophthalmus viridescens*.

REPRODUCTION

Age and size at maturity.—Lake (1936: 828) stated that all *Etheostoma f. flabellare* reach maturity and spawn in the spring of the year following that of hatching. Raney and Lachner (1943: 237) found that the faster-growing individuals of *Etheostoma nigrum olmstedi* attain maturity and spawn when one year old, and all individuals are mature in the second year; and that some males and females of *Etheostoma longimanum* spawn at the age of one year, and all breed at two years of age. Raney and Lachner (1939: 164) reported that *Etheostoma*

maculatum reaches maturity and spawns at the age of two years. Lachner *et al.* (1950: 109) found that individuals of *Etheostoma z. zonale*, *E. variatum*, and *E. b. blennioides* are mature and spawn when two years of age; some *zonale* mature and spawn at one year. The Salmon Creek population of *blennioides* agrees with *E. f. flabellare* in that all males and females reach maturity and spawn in the spring of the year following that of hatching. At this time males range in standard length from 39.0 to 51.5 mm. (mean = 45.7 mm.), and females range from 35.0 to 46.0 mm. (mean = 41.2 mm.).

In preliminary phases of this investigation, the bright coloration of both sexes of age-group I (Hubbs, 1943: 260) in the spring of the year suggested maturity. Further, the abdomens of the small females of this age-group appear distended with ripening eggs. Examination of ovaries of these females prior to, and after, the spawning season indicates that spawning occurs in this age-group. Finally, repeated spawnings of the age-group I fish in the laboratory demonstrated maturity in the first year. No young male or female was ever observed that had failed to attain maturity at this time.

Sex dimorphism.—Sexual dimorphism is most apparent in coloration and pattern of body markings. Adults can be separated on these bases in living or freshly killed material. Lachner *et al.* (1950: 95–96) pointed out the differences between the sexes in such characters as longer anal papillae in the female, larger body size in the male, faster growth rate in the male, and differences in the sizes of the various fins. Growth studies in the Salmon Creek population support these contentions regarding body size and growth rate. The peculiar black-tailed condition of large males (p. 142) may be a secondary sexual character, for this phenomenon has not been observed in females. The blackening of the males during the spawning season is a sexual dimorphic characteristic in the johnny and fantail darters.

Juveniles cannot be sexed, except by dissection methods, until six or eight months of age (January or February). At that time, males possess an orange-rufous band at the base of the first dorsal fin with a suggestion of one on the second, but females have only a suggestion of it on the first. The males are also developing the banded pattern of the adult at this period.

Sex ratio.—Lachner *et al.* (1950: 96–97) concluded that a significant departure from a 1:1 sex ratio exists in a population of *E. b. blennioides* in French Creek of the Allegheny River system, Pennsylvania. The specimens used in that study were collected on October 5 and 8, 1947. To afford a comparison with that work Salmon Creek specimens collected from October 1, 1949 through January 4, 1950 were used. The Salmon Creek data are treated and tabulated in a manner similar to that used by Lachner and his colleagues as shown in Table 3.

Collection group 1 in Table 3 represents the fish collected from October 1 through November 30, 1949; collection group 2 represents specimens collected December 29, 1949 and January 4, 1950. The specimens are arranged within these collection groups according to age-group. These age-groups do not strictly follow the suggestion of Hubbs (1943: 260), because the age of the January 4, 1950 specimens is rated as though they were collected December 31, 1949. The

TABLE 3

The probability for values of chi-square that a significant departure from a 1:1 sex ratio occurred in specimens of Etheostoma b. blennioides *of various age-groups from Salmon Creek, Lake Ontario drainage, Monroe County, New York*

Specimens of collection group 1 taken from October 1, 1949 through November 30, 1949; specimens of collection group 2 taken December 29, 1949 and January 4, 1950.

Coll. Group	Age-Group	No. of Spec. M.:F.	Sex Ratio M.:F.	Chi-Square	P
	0	26:29	1:1.12	0.164	.70–.50
	I	19:13	1.46:1	1.125	.30–.20
1	II	22:36	1:1.64	3.379	.10–.05
	III	3:1	—	—	—
	IV	0:1	—	—	—
Group 1 total		70:80	1:1.14	0.667	.50–.30
	0	17:18	1:1.06	0.029	.90–.80
	I	33:12	2.75:1	9.800	.01–.001
2	II	28:33	1:1.18	0.410	.70–.50
	III	1:4	—	—	—
Group 2 total		79:67	1.18:1	0.986	.50–.30
	0	43:47	1:1.09	0.178	.70–.50
	I	53:26	2.04:1	9.228	.01–.001
1 & 2	II	50:69	1:1.38	3.034	.10–.05
	III	4:4	—	—	—
	IV	0:1	—	—	—
Groups 1 & 2 total		149:147	1.01:1	0.014	.95–.90

chi-square value of the sex ratio, with the probability of that value, has been given for each age-group in each collection group, for the total number of specimens in each collection group, and finally, for the total number of specimens in both collection groups.

Lachner *et al.* (*loc. cit.*) reported a general excess of males in each age-group, and in age-groups 0, II, and III the probability of such ratios is below the 5 percent level. Such an excess of males is not indicated in Salmon Creek. Only in age-group I do the males exceed the number of females. In age-group I of collection group 2 the probability of the chi-square value for a ratio of 2.75 males to 1 female is below the 1 percent level. When the collection groups are combined, the age-group I specimens are in the ratio of 2.04 males to 1 female and the probability of the chi-square value for this ratio is less than 1 percent. In all other ratios the chi-square values have probabilities that could be expected as the result of chance. The combined specimens of both collection groups, regardless of age, give a chi-square value of 0.014. Such a value indicates that in a population where a sex ratio of 1:1 exists, a ratio such as that obtained (149 males to 147 females) could be expected 90 to 95 percent of the time. On the

basis of these data from Salmon Creek a sex ratio of 1:1 is indicated for the greenside darter.

It should be pointed out that a value of 14.907 is obtained if the chi-square values for age-groups 0, I, and II of both collection groups are totaled. Such a value at 6 degrees of freedom has a P value of 0.05–0.02. Closer scrutiny of that total chi-square value shows that age-group I specimens of collection group 2 contribute a value of 9.800. Thus, the other five contributors total a chi-square value of 5.107; this value at 5 degrees of freedom has a P value of 0.50–0.30. The preponderance of males in the age-group I specimens may be a reflection of the winter month distribution of age-group I females (pp. 144–145). Such females in quiet water stretches could easily be missed, because the same small seine as that used in the riffles was used there, where it would be an inefficient fish collector.

Raney and Lachner (1943: 233) found the sex ratio in *Etheostoma nigrum olmstedi* to vary considerably in different localities. Perhaps the differences in the results of the French Creek (Lachner *et al.*, 1950) sex ratio studies and those above may be attributable to this geographical phenomenon as well as to vastly different habitats.

Lachner *et al.* (*op. cit.*: 107) observed that in those darters in which the breeding habits are known, there is no consistency between nest-guarding activities and the tendency for males to exceed females in number. Among the nest-guarding species, Lake (1936: 828) reported more than twice the number of males to females in *E. f. flabellare*, and Raney and Lachner (1939: 163) found a ratio of 1.8 to 1 in favor of the males in *E. maculatum*. No nest-guarding is indicated in *E. b. blennioides* in Salmon Creek, and there the sexes appear to be present in equal numbers.

Fecundity studies. Distribution of ova-diameters in the ovaries.—When the life history of a fish is unknown, Hickling and Rutenberg (1936: 311–18) suggested that a study of the distribution of ova sizes in the ovaries may afford information concerning reproductive activities of the species. Harrington (1948: 83–92) demonstrated the value of such a method with the cyprinid *Notropis bifrenatus*. Carbine (1944: 128–33) studied the distribution of ova-diameters in the ovaries of *Esox lucius*, to determine the history of the developing ova and to check the assumption that all ova that are to be spawned in one year become distinguishable during the seasonal development from small immature ova. Carbine (*op. cit.*: 132–33) also measured the ova from one specimen of *Esox vermiculatus*. A similar study was made with the greenside darter to obtain information about, (1) the number of eggs spawned by females of different ages, (2) the age of females at maturity, and (3) the length of the spawning season.

Darters are spring spawners, as far as is known. In the spring, the brightened coloration of both sexes and the distended abdomens of the females indicate that this is true of the greenside darter. Gross examination of the ovaries from specimens of different size collected in February and March (either preserved or fresh) showed them to be full and compact, an indication that spawning had not yet occurred for the respective season. To determine the conditions existing in

the ovaries of late winter females, microscopical examinations were made of the ovaries of preserved females of age-groups I, II, and III (Hubbs, 1943: 260) collected in Salmon Creek during March. These specimens had been killed in 10 percent formalin and kept in that solution for 3 days, then placed in water for 3 days and in 50 percent ethyl alcohol for 5 days. They were then placed permanently in 75 percent ethyl alcohol. Each ovary of each female was cut transversely into three regions, an anterior, a central, and a posterior region. These pieces were embedded in paraffin and sectioned. Standard cytological techniques were employed, but cedar oil instead of xylol was used as a clearing agent (Clark, 1925: 5). Sections 10 microns thick were stained in hematoxylin and counterstained in eosin.

No differences in the distribution of ova sizes are apparent among the three different ovary regions or between ovaries of the same fish. The larger females usually possess more large ova (diameter > 1.0 mm.) than the smaller females. In all sections the smaller ova are most abundant near the ovigerous lamellae but also occur in the interstices between larger ova. In all sections of ovarian tissue examined the ova ranged in diameter from less than 0.02 to 1.90 mm. The greater mass of the ovaries is composed of ova exceeding a diameter of 0.20 mm. Ova sizes between 0.10 and 0.20 mm. diameter are scarce, but ova less than 0.10 mm. in diameter are very numerous. The cytoplasm in ova of less than 0.10 mm. diameter stains a deep red, and the nucleus is visible as a rounded blue area, but yolk is not in evidence. Relatively few ova are observed in the diameter range of 0.10 to 0.20 mm., and in these the yolk first appears as red globules when a diameter of 0.10 to 0.12 mm. is reached; at 0.14 mm. the yolk is apparent as a dark red mass surrounded by more lightly stained cytoplasm, and at 0.16 mm. the yolk stains a dark red, obliterating the cytoplasm.

Because preliminary microscopic examinations showed that the total number of ova per female would amount to several hundred at most, it was decided to count all those developing rather than some fraction. Six females were selected for the study of the distribution of ova-diameters, three specimens representing age-groups I, II, and III collected April 10, 1947, from Salmon Creek, and three similarly-aged specimens collected April 20, 1935 from the Shenango River, Mercer County, Pennsylvania. The sides of all six specimens were distended with ova, and after dissection the ovaries of each appeared full and compact. All measurements were made with a binocular dissection microscope to the nearest ocular micrometer division (1 div. = 0.02017 mm.) with the excised ovary contained in a 75 percent ethyl alcohol solution. Comparison of the ovaries of freshly caught specimens with those from preserved females show no evidence of distortion in the preserved ova. The method of measurement employed is that of Clark (1925: 4–5) who established its statistical validity.

In Table 4 is shown the distribution of ova-diameters of the six females. Measurements are grouped at five-micrometer intervals in the first column, converted to appropriate millimeter class intervals in the second, then placed under the proper drainage system and age-group. All ova less than 0.10 mm. diameter are included in the first row of the table. The plus (+) signs after each ova count

TABLE 4

Frequencies of ova-diameters, at five micrometer intervals, from both ovaries of six pre-spawning season female Etheostoma b. blennioides *segregated by age-group and drainage*

(1 micrometer interval = 0.02017 mm.)

Ova-Diameters		Salmon Creek Age-Group			Shenango River Age-Group		
Micrometer Divisions	Millimeters	I	II	III	I	II	III
1–45	0.02–0.10	137+	120+	220+	97+	140+	240+
6–10	0.12–0.20	—	—	21	6	5	14
11–15	0.22–0.30	32	62	93	18	66	78
16–20	0.32–0.40	110	110	200	38	116	159
21–25	0.42–0.50	122	78	234	89	153	246
26–30	0.52–0.61	134	123	248	79	114	220
31–35	0.63–0.71	56	115	132	37	80	202
36–40	0.73–0.81	20	90	114	22	54	136
41–45	0.83–0.91	10	56	78	39	44	87
46–50	0.93–1.01	—	40	24	27	36	52
51–55	1.03–1.11	—	19	126	4	12	41
56–60	1.13–1.21	—	36	135	9	42	54
61–65	1.23–1.31	—	40	30	16	48	38
66–70	1.33–1.41	—	4	3	13	10	5
71–75	1.43–1.51	—	—	—	7	—	24
76 80	1.53–1.62	—	—	—	—	—	55
81–85	1.63–1.72	—	—	—	—	—	41
86–90	1.74–1.82	—	—	—	—	—	16
91–95	1.84–1.92	—	—	—	—	—	4
Totals		484	773	1438	404	780	1472

in the first row indicate that many small yolkless ova less than 0.02 mm. diameter were present but not counted. The dashed line between the first and second rows indicates that the ova counts of the first row have not been included in the total ovary count of each column.

From the total counts it appears that older, larger females possess more yolked ova in the early spring than the younger smaller females. In both drainages, as represented by these six specimens, an age-group III female possesses about twice the number of ova with yolk as an age-group II female, and about three times the number possessed by an age-group I female. It is also apparent that older and larger females possess more ova of a diameter exceeding 1.0 mm. at this time.

In Figures 1, 2, and 3 the frequency distributions of ova-diameters of Table 4 are graphically shown. In each figure, females of the same age-group but of different drainages are compared, the specimen from Salmon Creek at the top, that from the Shenango River at the bottom. All ova less than 0.10 mm. in diameter are represented at the extreme left of each graph as a barred column. Interpreted in the light of the findings of Hickling and Rutenberg (1936: 311–18)

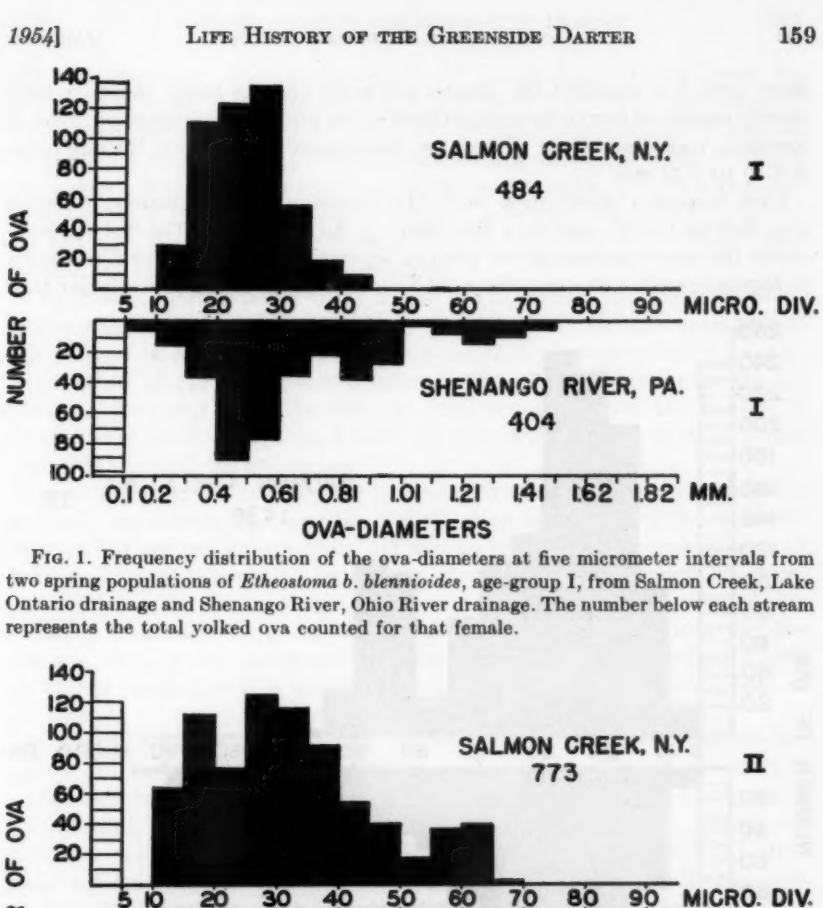

FIG. 1. Frequency distribution of the ova-diameters at five micrometer intervals from two spring populations of *Etheostoma b. blennioides*, age-group I, from Salmon Creek, Lake Ontario drainage and Shenango River, Ohio River drainage. The number below each stream represents the total yolked ova counted for that female.

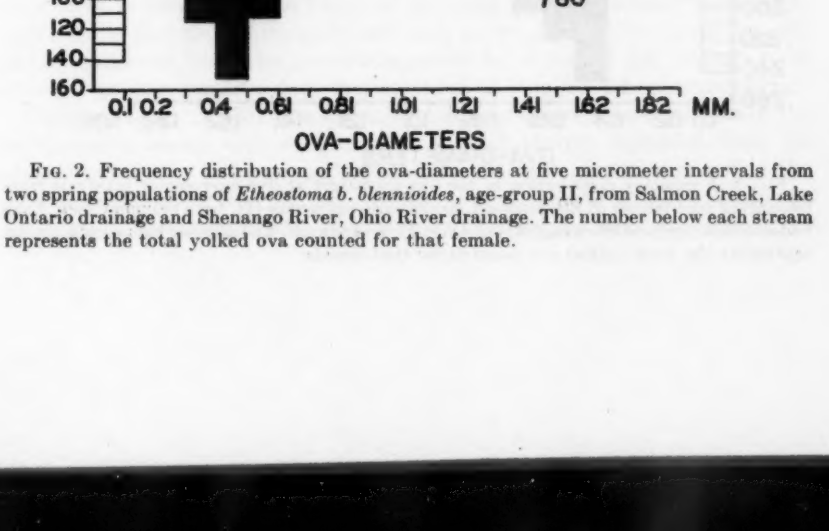

FIG. 2. Frequency distribution of the ova-diameters at five micrometer intervals from two spring populations of *Etheostoma b. blennioides*, age-group II, from Salmon Creek, Lake Ontario drainage and Shenango River, Ohio River drainage. The number below each stream represents the total yolked ova counted for that female.

these small ova represent the general ova stock and the larger ova have been sharply separated from it to undergo maturation prior to being spawned. In each specimen, regardless of age or drainage, this separation occurs in the size range of 0.10 to 0.20 mm.

Each frequency distribution (with the exception of the age-group I female from Salmon Creek) exhibits a bimodality in the yolked ova. The first mode includes the ova-diameters at the point of separation from the general ova stock to approximately 1.00 mm.; the second mode consists of those ova larger than

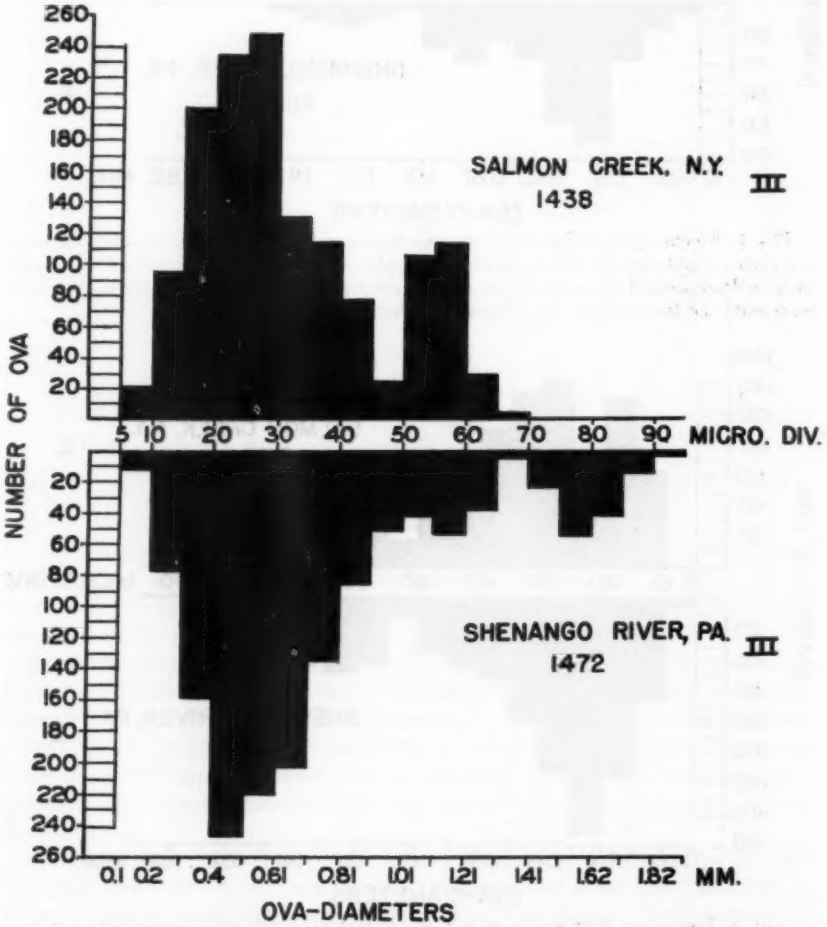

FIG. 3. Frequency distribution of the ova-diameters at five micrometer intervals from two spring populations of *Etheostoma b. blennioides*, age-group III, from Salmon Creek, Lake Ontario drainage and Shenango River, Ohio River drainage. The number below each stream represents the total yolked ova counted for that female.

1.00 mm. diameter. (For the age-group III female from Salmon Creek the second mode begins at a diameter of 0.91 mm., and that for the age-group III female from the Shenango River begins at 1.31 mm.) This bimodality suggests the possibility that during the approaching spawning season the different females might spawn only those ova contained in the second mode. If such is the case, the age-group I, II, and III females of Salmon Creek would have spawned 0, 139, and 318 eggs, respectively; the females from the Shenango River would have spawned 76, 148, and 330 eggs, respectively.

The greenside darter is one of the largest of the darters. The literature of the Etheostomatinae indicates that the larger species produce the greater number of eggs (Tables 10 and 11). On this basis it might be expected that *blennioides* would spawn all the ova separated from the general ova stock during the approaching spawning season because the total ova counts for the females of different ages as shown in Table 4 more nearly approximate the number of eggs commensurate with the species size.

The distribution of ova-diameters in the ovaries of the six selected females is essentially the same in all, regardless of age or drainage. If, during the approaching spawning season, an age-group III and an age-group II female spawn all the ova contained in the batch found to be separated from the general ova stock, then it is indicated that an age-group I female should spawn all its ova that are contained in the same size groups. If the bimodal tendency in the graphs signifies that only those ova of the second mode are spawned, then it is indicated that not all age-group I females spawn in the spring of the year following that of hatching.

In regard to the problem of length of spawning season in *blennioides* the frequency distribution study of ova-diameters provides suggestions, especially if the known fecundity data from etheostomine literature are compared. Figures 1, 2, and 3 show that in each female the ova are withdrawn from the general ova stock in a sharply separated batch. Hickling and Rutenberg (1936: 311, 314) postulated that in those fishes with a long spawning period the withdrawal of ova from the general ova stock for subsequent development and spawning within that period would be a continuous process, but in the case of fishes with a short spawning season, the ova to be matured and spawned would be withdrawn in a single batch sharply separated from the general ova stock. These authors found this postulate supported by certain marine fishes with known life history. Harrington (1948: 92) questioned the degree to which this criterion of ova development in relation to the length of spawning season is applicable to fresh-water fishes, because so little published data are available. Harrington (*loc. cit.*) further suggested that a long spawning period for a marine fish appears to be of much greater duration than that regarded as long for a fresh-water form.

Carbine (1944: 123–33) found that in *Esox lucius* the developing ova in the pre-spawning season were withdrawn in a batch sharply separated from the general ova stock, and he considered the 3- or 4-week spawning period of the pike to be short. Carbine (*loc. cit.:* 132–33) studied the ova development in one specimen of *Esox vermiculatus* and, because intermediate sizes of ova occurred

between the minute general ova stock and the "maturing ova" he considered this type of development to be different from that found in *Esox lucius*. The spawning season of *E. vermiculatus* coincides with or overlaps that of *E. lucius*.

It is suggested here that ova development in both esocids is essentially the same; that is, ova to be matured and spawned in the approaching season are withdrawn sharply from the general ova stock prior to the breeding season. The graphs of *E. lucius* and *E. vermiculatus* (Carbine, 1944: Figs. 3 and 4) compare favorably with those of the herring and haddock (Hickling and Rutenberg, 1936: Fig. 1), respectively. Harrington (1948: 88–92) found that in the cyprinid, *Notropis bifrenatus*, the developing ova are withdrawn in a continuous manner, and he considered the spawning period of 7 or 8 weeks to be long for a fresh-water fish. The manner of ova development in the greenside darter is similar to that of the haddock and the mud pickerel, *Esox vermiculatus*. On the basis of ova-diameter distribution in the pre-spawning season alone, the length of the spawning period for the greenside might be forecast as up to 5 weeks in length. It was determined by different methods that the length of the spawning season in the greenside extends from 5 to 9 weeks and thus approaches the long spawning season of *Notropis bifrenatus*. As pointed out by Harrington (1948: 92), not enough published data regarding fresh-water fishes with a long spawning period are available as yet to establish the relationship of ova development to length of spawning season in fresh-water fishes.

Information concerning the length of time required by the greenside darter to spawn might be obtained by a comparison of fecundity data among other percid darters where such is available. If all the ova in the batch sharply separated from the general ova stock are spawned in one season, then the problem becomes a question of how much time is required for a female to develop an ovum of 0.10 mm. diameter to one of spawning size which was indicated to be in the range of 1.90 to 2.0 mm. diameter. Lake (1936: 824) found that *Etheostoma f. flabellare* deposited eggs of 2.3 mm. diameter in batches of about 45 approximately five times during the spawning period of 8 or 9 weeks. Here a relatively small number of eggs of large size required a long period to be deposited. Raney and Lachner (1939: 162) reported that a large *Etheostoma maculatum* spawned about 400 eggs of 2.0 mm. diameter on two occasions in a period of about 5 weeks. Here a large number of eggs of large size requires a shorter time to be deposited than in the case of *E. f. flabellare*. J. Petravicz (1936: 81–82) found that *Etheostoma punctulatum* spawned 30 eggs of 0.7 mm. diameter daily for a week. Here a small number of small eggs is deposited in a short time. If all the ova larger than 0.10 mm. in diameter in the ovaries of pre-spawning season females of *blennioides* are spawned in one season, then the greenside spawns a large number of large eggs. The period of time required for these eggs to be deposited could be estimated as longer than the period of either *flabellare* or *maculatum*.

The study of the distribution of ova-diameters in *blennioides*, coupled with fecundity data in the literature of the Etheostomatinae, thus afford valuable suggestions regarding the three problems cited at the beginning of this section. Other information is also provided. The number of immature ova of diameter

less than 0.10 mm. though abundant do not appear to outnumber the ova of greater diameter. It is probable that a female about to spawn for the first time does not have the 2200 immature ova at that time that would be required for the following two spawning seasons (Table 4, first row). Thus, as Carbine (1944: 131) found for *Esox lucius*, it is indicated that new ova develop during the adult life of *Etheostoma b. blennioides*.

Ova counts prior to, and after, the spawning season.—To obtain other indirect evidence concerning the number of eggs spawned by females of different age and the age at maturity of females, an examination of the ovaries of females of different age-groups prior to, and after, the spawning season was necessary. In 1948 the spawning season extended from May 5 through June 11. (The study of the distribution of ova-diameters indicated a longer period than this.) Ten preserved females, representing age-groups I, II, III, and IV collected in Salmon Creek in the first week of April, 1948 were dissected, and an actual count of the ova of both ovaries of each fish was made. All ova of 0.10 mm. diameter or larger were counted. Although both left and right ovaries of the greenside darter appeared to be of equal size, the ova counts were tallied for each for purposes of comparison.

The results of the ova counts from the ten ripe females are shown in Table 5. Three specimens each of age-groups I, II, and III and one specimen of age-group IV are represented with their standard lengths, the actual ova count for left and right ovaries, the actual ova count, the estimated total ova count for the fish based on the actual count of either left or right ovary alone, and the percent error involved in that estimate. The ova counts are in very close agreement

TABLE 5

Direct and estimated counts of ova in both ovaries of ten, preserved, ripe female Etheostoma b. blennioides *segregated by age-groups*

Ova estimates for each specimen based upon doubling the actual count of either ovary alone and the percent error given in such estimates. Specimens collected during the first week of April, 1948 in Salmon Creek, Monroe County, New York.

Age-Group	S.L. mm.	Direct Ova Count			Estimated Count		Percent Error
		L.Ov.	R.Ov.	Tot.	L.Ov.	R.Ov.	
I	41.5	248	236	484	496	472	2.5
	43.0	199	205	404	398	410	1.5
	43.0	259	251	510	518	502	1.6
II	51.0	381	392	773	762	784	1.4
	53.0	418	381	799	816	762	2.4
	55.0	379	401	780	758	802	2.8
III	60.0	745	717	1462	1490	1434	1.9
	63.5	742	730	1472	1484	1460	0.8
	66.0	804	762	1566	1608	1524	2.7
IV	72.5	926	901	1832	1852	1812	1.1

with the counts made in the previous spring during the study of the distribution of ova-diameters. The differences between ova counts for the left and right ovaries are relatively small, and, as shown in the last column, if the total count of ova for any female is based upon doubling the count from either ovary, an error of less than 3 percent is involved. It appears justified in the future to compute total pre-spawning season ova counts of *blennioides* from the count in one ovary.

Within three to six days after the end of the spawning season females representing age-groups I, II, and III were collected. At this time the ovaries are greatly reduced in size and only yolkless ova are present. There are no ova as large as 0.10 mm. diameter. These conditions are the same in all females (16) examined. These findings support the indications of the study of the distribution of ova-diameters, namely, that at least all ova of 0.10 mm. diameter or larger, prior to the spawning season, mature to be spawned in that approaching spawning season. Whether any yolkless ova mature during the spawning season remained to be determined. These post-spawing season ovary examinations further support the ova-diameters work in that no ova with yolk was found in the six age-group I females. It was thus strongly indicated that all females attain maturity and spawn in the spring of the year following that of hatching.

Count of eggs spawned in rearing troughs.—In the spring of 1948 it was discovered that greenside darters could not only be kept alive successfully in the laboratory, but the fish would spawn. Further, the spawning season for the species had been delimited so that in 1949 an experiment was set up to provide direct evidence regarding the three problems originally approached through a study of the distribution of ova-diameters. This experiment was also set up to provide information regarding the age at maturity of males.

Greenside darters of both sexes representing age-groups I, II, and III were brought into the laboratory well in advance (April 2) of the beginning of the spawning season (April 13). Two, standard, hatchery-type, rearing troughs were divided into three compartments each. A compartment was approximately 21 inches in length, 14 inches in width, and 7 inches in depth. A rock from Salmon Creek covered with *Cladophora* was placed in each compartment. A male and a female darter of comparable age were also placed in each compartment so that age-groups I, II, and III were each represented twice among the six compartments. Conditions of water flow and aeration were the same as described below (pp. 171–172). *Enchytraeus* worms were supplied constantly as food in each of the compartments from a standard, floating enchytrae feeder. The known spawnings by earlier captive fish had occurred during hours of darkness, therefore the necessary weekly cleanings were performed during daylight periods. Whenever it was necessary to replace an alga-covered rock, the alga of the replacement rock was searched for any chance wild eggs. Each week of the experiment the males were exchanged for fresh, wild males to insure that the females were attended constantly by potent males. Each morning each alga-covered rock was taken from its compartment and examined carefully for eggs. If eggs were present, they were collected, counted, and isolated; the rock, if

TABLE 6

The dates of spawning and number of eggs spawned per spawning night in the laboratory by five selected female Etheostoma b. blennioides *Raf. of different age-groups during the breeding season of 1949*

Age-Group	I				II				III	
Stand. L.	41.0 mm.		44.0 mm.		52.0 mm.		54.0 mm.		62.0 mm.	
No. of sp. nights	Date	No. of eggs	Date	No. of eggs	Date	No. of eggs	Date	No. of eggs	Date	No. of eggs
1	5/11	52	5/ 4	42	4/18	54	4/29	48	4/13	67
2	16	44	6	61	20	68	5/ 2	54	15	84
3	19	32	8	23	22	94	4	106	17	72
4	22	16	9	19	26	113	7	120	19	92
5	24	52	13	47	5/ 1	110	9	118	22	192
6	28	40	17	52	4	96	10	94	25	132
7	6/ 2	38	21	33	8	16	11	86	28	126
8	5	54	24	28	12	110	17	24	30	161
9	7	2	29	62	15	74	18	82	5/ 3	140
10	11	42	6/ 2	16	18	48	22	10	6	131
11			6	32	20	36	24	62	9	122
12							27	10	13	86
13									17	73
Total no. eggs sp.....		372		415		819		814		1478
Av. no. eggs per sp. night.....		37.2		37.7		74.5		67.8		113.7

still serviceable, was replaced in its respective compartment. In late afternoon the alga was again examined, but the rock was left in place. The experiment was conducted from April 2 through June 14, 1949. At that time the females were sacrificed for an ovarian examination.

At the start of the experiment the water temperature was 47°F. By April 8 the temperature had risen to the critical point of 51°F. and afterwards continued to rise until at 54°F. during the night of April 12–13 spawning first occurred. In Table 6 are shown the spawning records of the six females during this experiment with the total number of eggs per female and the average number of eggs per female per spawning night.

The age-group III females did not spawn after May 17; the age-group II females did not spawn after May 20 and 27, respectively; the age-group I females spawned last on June 6 and June 11, respectively. The older fish had lost a considerable amount of their bright coloration, and, because the end of the breeding season was approaching, it was decided to sacrifice the experimental females to reduce any chances of the resorption of mature ova and thus their consequent loss in the accounting. When the females were sacrificed on June 14, it was established that all females, with the exception of that age-group III fish which spawned a total of 1142 eggs, possessed only immature ova of a diameter of less than 0.10 mm. (Within a few days the end of the wild breeding season was

established as June 12, hence the experimental fish had spawned in the laboratory almost to the day that the wild fish did.) In the age-group III female which failed to spawn all its mature eggs, the left ovary was found to contain 316 mature ova ranging in size from 1.83 to 1.89 mm. in diameter; all remaining ova were immature and less than 0.10 mm. in diameter. Why the 316 ova were not spawned is not known, but perhaps the duct for the left ovary became defective as the spawning season progressed. It appears unlikely that these 316 ova would have been spawned if the fish had not been sacrificed, because 28 days had elapsed since the last spawning, and such a long period between spawnings did not occur in any of the experimental fish. The 316 ova should be added to the 1142 spawned by this female to give a total of 1458 eggs.

From this experiment an exact count of the eggs spawned by females of different age-groups could be made. The totals in Table 6, age-group for age-group, compare very favorably with the indicated totals of the ova-diameters distribution study and the ova counts prior to, and after, the spawning season. The pre-spawning season ova counts appear to be valid estimates of egg production in the greenside darter. From the different methods utilized, the range in number of eggs spawned by females of different ages is as follows: age-group I (females spawning for the first time), 372 to 510 eggs; age-group II, 773 to 819 eggs; age-group III, 1438 to 1566 eggs; and age-group IV (based only on the pre-spawning season ova count of one specimen), 1832 eggs.

The data in Table 6 show that an age-group III female spawned first on April 13; the next individual to spawn was an age-group II fish on April 18. The other age-group III female spawned on April 22 and the other age-group II female on April 29. The first age-group I female to spawn did so on May 4 followed by the other on May 11. Thus the older fish spawned earlier in the season. Both age-group III females spawned last on May 17; the age-group II fish spawned last on May 20 and 27, respectively, but the age-group I females last spawned on June 6 and 11, respectively. These dates of spawning suggest that the older females are the first to spawn in the spawning season and the first to finish. The age-group I females spawned 10 and 11 nights, at a rate of 37.2 and 37.7 eggs per spawning night, over a period of 30 and 32 days, respectively. The age-group II females spawned 11 and 12 nights, at a rate of 74.5 and 67.8 eggs per spawning night, over a period of 32 and 28 days, respectively. The age-group III female that spawned all the mature ova spawned 11 nights at the rate of 113.7 eggs per spawning night over a period of 34 days. The age-group III female that failed to spawn all the ova complement spawned 11 nights at the rate of 103.8 eggs per night over a period of 25 days. It appears that from 4 to 5 weeks are required for an individual female to spawn all its eggs, regardless of the age of the female. The older females spawn their greater number of eggs at a faster rate per spawning night then the younger ones.

Regarding the age at maturity of male greenside darters, the experiment established the fact that males, like females, attain maturity and spawn in the spring of the year following that of hatching. Age-group I females in the experiment were constantly attended by age-group I males and every male used (10) suc-

ceeded in spawning. These data are further supported by the fact that in 1950 many males of age-group I were used to spawn with females of the same age to obtain eggs for embryological studies. The only age-group I males that failed to spawn in the laboratory were some used in early June and subsequent examination showed these individuals to be spent.

Spawning habits. Time of year.—Observations based upon four successive spawning seasons indicate that the spawning period for *blennioides* in Salmon Creek occurs from the second week in April through the second week of June with the height of the spawning occurring in May.

The spawning season of the greenside was delimited during the spring of 1948 by means of daily checks at so-called "egg-stations". Seven egg-stations were located at suitable riffle sections along the stream between the Barge Canal and the confluence of Tributary No. 2. Beginning the last week of March, at least two of these stations were carefully searched for eggs daily. By this method, spawning was found to have occurred first on May 5, and by the same method but at intervals of three or four days, spawning was found to have ceased on June 11. Using the same method (but at a two day interval in late March and early April) the initiation and cessation dates of spawning for 1949 and 1950 were found to be April 13 to June 12, and April 27 to June 10, respectively. The breeding season of 1949 was considerably longer than either that of 1948 or 1950. In the area under consideration the spring of 1948 was characterized by the rapid runoff of water from late winter snow and frequent rainstorms; the resultant flash-floods scoured the stream bed to present other than normal conditions. In the spring of 1950 the weather was unusually cold, and the water temperature in Salmon Creek did not reach 50°F. until April 18. This day was immediately followed by six days of cold weather (air temperature readings were between 42° and 48°F.). The spring of 1949, however, witnessed climatic conditions which more closely approached the ideal, that is, slow runoff of water from snow, a moderate spring rainfall, and a gradual rise in the air temperature through late March and early April (Fig. 4). Although the initiation of spawning was apparently delayed in the years 1948 and 1950, the dates of cessation of spawning for all three years are remarkably consistent. The 1949 spawning season is indicated to be the most typical when all factors are considered.

Time of day.—Greenside darters appear to be primarily nocturnal spawners. There were only two occasions when spawning occurred in the laboratory during daylight hours. On May 25 and 27, 1950 spawning occurred at approximately 6:00 p.m. and 6:30 p.m. (e.s.t.). The spawning acts on these occasions were not witnessed, but 2- and 4-cell cleavage stages of the eggs were obtained. These daylight spawnings may have been due to laboratory conditions. In the stream, such early stages were never found, but the many hundreds of eggs collected were always taken during the daytime from mid-morning to late afternoon.

Effect of water temperature upon spawning.—Water temperature plays an important role in the spawning activity of the greenside darter. The critical water temperature for the initiation of spawning appears to be 51°F., but that temperature, or a higher one, must be maintained for a period of a few days.

In each of three spawning seasons such a phenomenon was observed. The readings of water temperature were made during morning hours.

In 1948, although the water temperature reached 57°F. on one day (April 20) prior to the date of first spawning (May 5), no temperature higher than 50°F. prior to May 1 was maintained for longer than approximately 36 hours. In this spring the observed temperatures for the period April 28 through May 5 were as follows: 48°, 48°, 49°, 50°, 51°, 51°, 54°, and 55°F. In 1949 the water temperature gradually rose to 50°F. (April 10), and subsequent readings for the period April 11 to April 13, the first spawning, were as follows: 51°, 53°, and 55°F. In 1950 the water temperature often rose above the 51°F. level, but these temperatures were not maintained longer than 24 hours until April 24. The temperature readings for April 24 to April 27, the first spawning, were as follows: 51°, 54°, 55°, and 53°F.

After the initiation of spawning, a drop in the water temperature below 51°F. can cause the cessation of egg-laying. In 1950 spawning continued nightly after its initiation on April 27 through the night of May 7–8. On May 8 a heavy downpour of cold rain decreased the water temperature in Salmon Creek from 57° to 48°F. On the morning of May 9 a few eggs that had been spawned the previous night were found at four egg-stations, but on the following day no newly-spawned eggs could be located. Within three days the water temperature had returned to 51°F., and on May 16 eggs that had been spawned on the night of May 15–16 were found in quantity at five different egg-stations. During this period, May 7 through May 16, breeding darters in the laboratory spawned continuously, but the water temperature (Lake Ontario water supply) in the troughs varied only between 53° and 57°F.

The effect of a drop in water temperature below 51°F. upon the spawning activity was demonstrated in the laboratory. In the three compartments of each of two rearing troughs a breeding pair of age-groups I, II, and III were placed, one pair to a compartment. In the first five days of occupancy (May 13 through May 17) the water temperature in the troughs varied from 54° to 56°F. at a rate of flow of one gallon per minute. During this time each of the pairs of fish spawned at least once. On the sixth day of occupancy the water flow was cut to a trickle and pieces of ice were introduced to one of the troughs. With no difficulty the water temperature was reduced to a level between 48° and 50°F. after three hours. Aeration was accomplished by the dispersion of air through carborundum breakers from standard laboratory air outlets.

The temperature range of 48° to 50°F. was maintained for six days, and during this time no spawning occurred. On the twelfth day of the experiment (May 24) no more ice was added, and the water temperature rose to 52°F. within 18 hours. The water flow rate was restored to one gallon per minute on May 25. Forty-two hours after the point where no ice was added the water temperature rose to 56°F. On May 26 with the water temperature at 58°F. the age-group II and III fish spawned 124 and 146 eggs, respectively. The following night the age-group I fish spawned 72 eggs. When the temperature was maintained between 48° and 50°F. in the experimental trough, the water temperature in the

control trough varied from 56° to 58°F. and the three pairs of fish there spawned without interruption.

The graph in figure 4 clearly shows the relationship of the spawning period of the greenside darter to the water temperature of Salmon Creek for the spring of 1949. It is not known whether a high water temperature could cause cessation of spawning. Such a phenomenon was not observed in the stream, and no experiments were conducted in the laboratory.

Location of the spawning site.—The spawning site of the greenside darter in Salmon Creek is a growth of the filamentous green alga, *Cladophora* sp., attached to the upper surface or sides of rocks, or the surface of sheets of bedrock, in the riffle sections of the stream. Usually the eggs are deposited at, or very near, the point of attachment of the algal strand to the rock. In those instances in which the eggs are found several inches or a few feet from the holdfast point, the egg-bearing strands of alga overlay rocks downstream from the holdfast rock; presumably the spawning act occurred on the alga over these downstream rocks. In the first and second weeks of spawning, eggs are found chiefly upon the large *Cladophora*-covered rocks located in the deepest and swiftest part of the riffles. Later, the eggs are found on alga-covered rocks in any part of the riffles, regardless of depth or rate of flow of the water. In figure 4 the estimated percent of riffle area covered by growths of *Cladophora* throughout the year (solid line) is

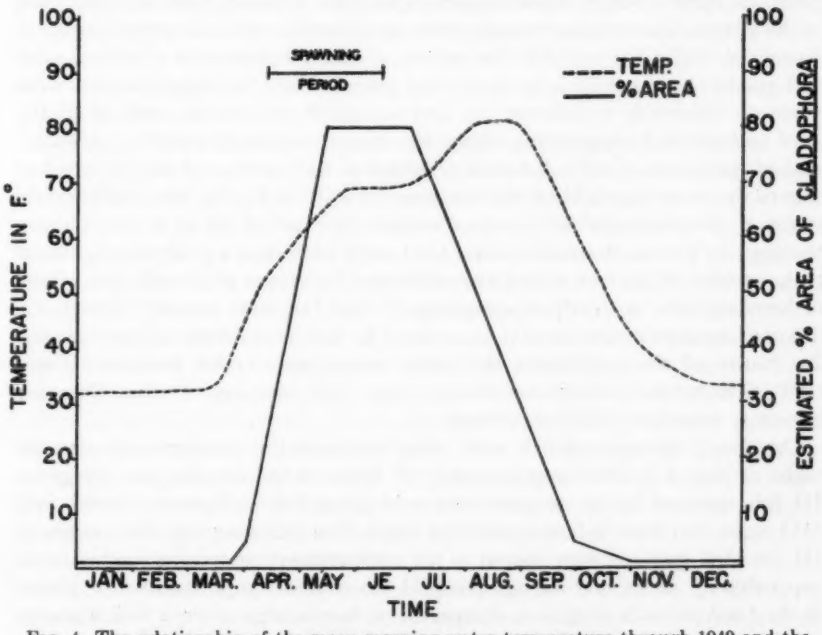

FIG. 4. The relationship of the mean morning water temperature through 1949 and the estimated percent of riffle area covered by *Cladophora* in Salmon Creek, Monroe County, New York. The spawning season of *E. b. blennioides* is indicated.

shown. The estimates were made purely by observations. This percent of riffle area covered by *Cladophora* growth increases rapidly from almost zero in late March to approximately 25 percent by the beginning of the spawning season in mid-April. During the spawning season the alga-covered area of the riffle increases to a maximum of 80 percent (Fig. 4). This maximum is maintained throughout the spawning season of the greenside darter and later into early July. At this time the area covered by the alga begins to decrease and by the first of November very few growths of alga can be found.

During three breeding seasons a search was made for spawning sites other than the above. This search included the sifting of sand and gravel, inspection of rocks, sticks, boards, logs, and associated vegetation chiefly *Anacharis*. No eggs were found in any situation other than *Cladophora*-covered rocks. From the literature, darters are known to deposit their eggs on a variety of objects. *Etheostoma nigrum* (Seal, 1892: 9), *Etheostoma f. flabellare* (Lake, 1936: 818), and *E. maculatum* (Raney and Lachner, 1939: 161) deposit their eggs on the undersides of stones. *Hadropterus maculatus* (W. Petravicz, 1938: 42) lays its eggs in shallow depressions of sand and gravel. *Etheostoma punctulatum* (J. Petravicz, 1936: 81–82) lays its eggs on submerged vegetation, on small objects in the water, or even on the sides of the glass aquarium.

In 1949 a simple experiment was conducted to determine whether breeding greenside darters would deposit eggs in situations different from the type used in the stream. Two rearing troughs were each partitioned into compartments of dimensions 31″ x 14″ x 7″. On the bottom of each compartment a layer of sand and gravel about one inch in depth was placed. The four compartments were made up differently as follows: the first contained a bare rock held up off the sand and gravel by supporting sticks; the second contained a portion of water-soaked barrel stave and a flat rock arranged in such a manner that the underside of the stave was held off the bottom; the third had a flat rock with several stems of *Anacharis* tied to its upper surface, one end of the rock raised above the sand by means of a small stone; the fourth contained a *Cladophora*-covered rock, one end of the rock raised above the sand by means of a small stone. Pairs of breeding fish, two each of age-groups II and III, were rotated through the different compartments to see if eggs would be laid on different objects. During the course of the experiment the water temperature varied between 55° and 64°F. Compartments were searched for eggs each morning. At that time any necessary transfers of fish were made.

On May 2 the pairs of fish were introduced into the compartments. On the night of May 4–5, after approximately 60 hours in the troughs, the age-group III fish spawned in the compartment containing the *Cladophora*-covered rock (114 eggs). No other fish spawned that night. The following day, the age-group III fish that spawned were moved to the compartment containing the bare rock supported by sticks, and the age-group II fish of that compartment were placed in the *Cladophora*-bearing rock compartment (a new alga-covered rock was supplied). That night, May 5–6, the age-group II fish spawned 43 eggs on the *Cladophora*, but no other spawning occurred. On May 7–8 the same age-group

II fish spawned again on the *Cladophora*-covered rock, but no other pair of fish spawned in the other compartments. The rotation of various pairs of breeding fish continued through the month of May in such a manner that each pair occupied each compartment for at least three days continuously on at least two separate occasions. Every pair of fish spawned at least once, but all spawning occurred in the one compartment, that containing the *Cladophora*-bearing rock. It appears that the greenside darter of Salmon Creek limits its spawning site to *Cladophora*-covered rocks. Another stream, Sandy Creek, approximately 7 miles west of Salmon Creek has luxuriant growths of *Cladophora* and the greenside darter also spawns on the alga there.

Investigations should be made in those streams containing the greenside darter, but no *Cladophora*. One such stream, briefly visited, was Wolf Run (Tributary No. 7), Allegheny River system, Cattaraugus County, New York. Here the vegetation of the riffles was chiefly the water moss, *Fontinalis* sp. On May 7, 1950 a cursory survey of two riffles in this stream failed to reveal any greenside eggs. The water temperature was 51°F. but perhaps this temperature had not been maintained long enough for spawning to occur.

Description of the spawning act.—The spawning act of the greenside darter has not been witnessed in the stream. The chances of observation of the act in the wild are exceedingly small. Apparently, spawning occurs only at night, beneath rapidly flowing water, over rocks, between the thick moving strands of *Cladophora*. Furthermore, the fish are very sensitive to the intensity of light, red or white, required for perception by humans. These attendant difficulties force the description of the spawning act to be written from observed activity within the laboratory.

During the known breeding season, sexually mature males and females were brought into the laboratory and placed in standard hatchery-type rearing troughs of dimensions 65″ x 14″ x 7″. Four such troughs were available. Above these four parallel troughs and at right angles to them was located a conventional head trough. Water entered the head trough at a maximum rate of two gallons per minute. The water supply was the same as that used by the city of Rochester, New York. A darter placed in such chlorinated water, with no attempt at aeration, succumbed within 24 hours when the rate of flow was at a maximum.

Satisfactory aeration was achieved quite simply. Water entering the head trough from the mains was made to run over an unpainted board 1″ x 7″ x 18″ before falling into the trough. Water entering the individual troughs from the head trough was made to fall upon a glass plate 7 inches square. The water in the head trough was further aerated by the introduction of air under pressure from three laboratory outlets using conventional carborundum breakers. These aeration methods operated continuously. Mortality was reduced by these simple precautions to less than 1 percent.

It was necessary to place screens over the troughs to prevent the fish from leaping out. Such screens hindered observation from above, and for that reason, as well as to eliminate shadows caused by an observer, a watertight, plate glass window ¼″ x 7″ x 22″ was installed in the side of one of the troughs in such a

manner that it occupied the center of one side of a compartment. The corners of this compartment on each side of the window were plugged with wooden blocks to prevent any observed fish from being out of sight of an observer. A 10-watt, red, photographic bulb (Mazda) used with a goose-neck lamp reflector produced sufficient light to allow observation at the window. The light was arranged so that the reflector rested directly upon the screen over the trough. Depending upon the level of water in the trough, the light source was located from three to six inches above the surface. During May, 1950 this red bulb shone continuously during the hours of darkness upon a *Cladophora*-covered rock contained in the windowed compartment. A square rock was placed against the far side of the compartment from the window in such a manner that the fish could not conceal themselves from the observer. This windowed compartment served as the stage for the enactment of the first witnessed spawning display of *blennioides*.

In the compartment were placed two pairs of adult darters, one pair of age-group II fish and the other, age-group III. The rock in the compartment at this time was roughly rectangular (1½″ x 8″ x 10″). The filaments of *Cladophora* did not exceed ¾ of an inch in length (previously clipped so as not to hinder the vision of the observer). The upper edges of the rock on both inflow and outflow ends overhung the lower edges slightly, thus affording a retreat for the fish, but these niches were visible to the observer. The side of the rock toward the observer presented an upward-sloping surface covered with a short growth of *Cladophora*.

The above pairs of fish had been in the compartment for four days and nights and no spawning had occurred. During the watch on the fifth night the two males began an activity not previously witnessed. At 1:15 a.m. the smaller male (age-group II) and the larger male (age-group III) darted swiftly over the rock, alternately, about every 15 to 25 seconds (left to right and right to left before the observer). These movements described an arc, the highest point of which was approximately 3 inches above the rock. On only two occasions did any part of either fish touch the rock during these movements, and then merely by brushing the tip of the tail against the rock. This behavior lasted for almost 10 minutes, after which both males came to rest upon the top of the rock, the larger male near the inflow edge facing the far side of the trough, and the smaller one in the center of the rock facing the observer. During this display the two females remained in their respective positions, the larger female (age-group III) in the far corner of the inflow end of the compartment facing the rock and the smaller one (age-group II) under the overhanging edge of rock on the outflow side facing the window.

These relative positions were held for about 6 minutes. At this time the smaller male moved slowly off the rock, turned right toward the outflow end of the compartment, and when about 2 inches from the partitioning screen turned sharply about to the right, darted swiftly up to the center of the rock, then shot straight up, breaking the surface of the water. Upon breaking the surface film this male collapsed and fell limply to the center of the rock. While the smaller male was

falling to the rock, the larger male moved from his position on the rock to the bottom of the trough toward the inflow end. About 2 minutes later this large male slowly turned about to the right and faced the rock. Holding this position for about 6 seconds the large male darted toward the center of the rock and the smaller male. When collision appeared unavoidable, the larger male shot straight up to the surface breaking water, just as the smaller male had done previously. The larger male then collapsed and fell to a point near the center of the rock. When the larger male shot up toward the surface, the smaller one moved slowly toward the outflow edge of the rock.

This peculiar performance by both males was staged alternately at 1- to 2-minute intervals for 12 minutes. During these displays the larger male never moved when the smaller male rushed toward the center of the rock prior to shooting up toward the surface, but only once did the smaller male remain in place when rushed by the larger. The positions of these fish after falling to the rock varied with the performance, and neither male appeared to discriminate in regard to which end of the compartment he moved prior to the turn-and-rush. On no occasion did the males touch each other. The females showed no reaction to this display of the males and remained in their respective places.

At 1:43 a.m., after a performance by the larger male, both males faced each other on the surface of the rock, with the long axis of their bodies parallel to the plane of the window, the larger male facing the outflow end of the compartment and the smaller male facing the inflow end. They were about 1½ inches apart. Both fish held the pectoral fins tightly against the body but pointing straight down toward the rock with the tips buried in the algal mass. In both males there was an almost imperceptible movement of the caudal fin which approached a vibration. This position was held for about 30 seconds, after which the bodies of both fish dropped to the rock simultaneously as they raised their pectorals to the normal position. Over a period of 25 minutes this type of action was observed nine times. On the second, third, and fourth occasions the elevated position was assumed by both males with a strong movement of the caudal fin and a forward sweep of the pectoral fins which were then pressed against the body of each fish, pointing straight down into the alga. During these three occurrences, the distance between the snouts of the males varied from approximately ½ inch to almost 2 inches, and the angle of the bodies to each other was not always 180 degrees. Upon falling to the rock after raising the pectorals, the two males remained in the center area of the rock. After falling from the fifth elevation, both males moved to opposite sides of the rock, the larger male to the far corner on the inflow side and the smaller male to the center of the outflow edge. Before assuming the sixth elevated position, both males faced each other and moved towards each other very slowly by a series of jerks until their snouts were about 1 inch apart. They then assumed the elevated position. After falling from this position, the males moved again to the same locations on the rock that they had assumed prior to the sixth elevation. The seventh occurrence was conducted in the same way as the sixth. At the eighth elevation the position of the bodies, as held by the pectorals, lasted for the longest interval, almost a full

minute, when the smaller male moved suddenly toward the larger, passing the left cheek of that male, then up and over the dorsum of same in the region of the caudal peduncle. The smaller male came to rest upon the bottom of the trough about 3 inches from the edge of the rock on the inflow side. At about the time that the caudal fin of the smaller male passed over the dorsum of the larger, the large male dropped to the alga. As far as could be observed the fish did not touch each other during this maneuver. The smaller male turned from his position in the inflow end of the compartment and faced in the direction of the larger male. With the same jerking motion observed previously, the smaller male approached the larger. At this point the larger male turned to face the smaller. When their snouts were about 1 inch apart, both elevated themselves, but only for about 20 seconds. After falling from the ninth and last elevated position, the smaller male moved to the screen at the outflow end, while the larger male remained in the center area of the rock where he had dropped.

Each of these nine elevated positions had occurred over approximately the central area of the rock. In all the actions, the smaller male remained on the outflow side of the compartment, except at the conclusion of the eighth elevation when a position on the bottom of the trough at the inflow end was assumed. In all the actions, the larger male kept to the inflow area of the rock or its central area. During the display there was no indication of pugnaciousness on the part of either male. The two females remained in their original positions. At 2:08 a.m. the smaller female moved from her position to a spot about midway along the sloping edge of rock nearest the observer. This situation lasted for about 3 minutes; then the larger male and the smaller female moved simultaneously from the rock, the female to the trough bottom about 2 inches from the window on the outflow side of the compartment and the male to the trough bottom at the inflow end of the compartment.

At 2:12 a.m. the larger female moved from her corner around the larger male to a point in front of the window on the inflow end. Pausing for a few seconds there she then darted toward the rock and came to rest upon the sloping edge, her venter in contact with the rock and her caudal fin touching the trough bottom. At the same time the larger male turned so that the long axis of his body was at right angles to hers with his snout about 6 inches from her caudal peduncle. About 2 or 3 seconds after turning, the large male moved to a point about 3 inches directly behind the female. This position was held for about 1 minute. The male then moved to within 1 inch of the female by means of his pectoral fins, his head slightly to the left of the female. This last inch of distance was covered with no perceptible fin movements. Slowly, with no fin movements, the male moved to a position alongside the female with the tip of his snout just behind the origin of her extended left pectoral. (Sometime during this maneuver the smaller female moved to the outflow end of the compartment near the partitioning screen; the small male remained near the screen partition.) For about 4 minutes the larger fish remained in the above position. Under the red light any color changes in the two fish could not be perceived, but both were visibly excited as evidenced by very rapid respiration and a slight quivering of the body

of each. The male rose slowly above the female in such a manner as to support his weight upon his bent caudal fin and upon the right side of his venter which was in contact with the left side of the female's dorsum. In such a crosswise position the right pelvic fin of the male was in contact with the dorsum of the female at the point between her dorsal fins, and the right pectoral fin of the male pressed down upon the female's first dorsal fin. As soon as contact was made with the male in this superior position, the female moved with short, jerking movements up the slope of the rock to the flattened upper surface. The male was carried along. During this movement both dorsal fins of the female lay flat against the dorsum, the first pressed down by the right pectoral of the male, the second apparently collapsed. Both dorsals of the male were erect. Both fish were facing away from the window, but the female turned slightly to the left so that a profile view of her head was possible. Up to this point little of the weight of the male rested upon the female and the latter was not pressed down into the alga. The slight quivering of both bodies was still apparent. As soon as the female ceased her forward motion, the male moved slightly up and forward in such a manner as to cross the female's dorsum in the region of her first dorsal fin and placed his snout above and behind her right cheek with his body bent downward so that his right side was in contact with her left side posteriorly. The right side of the caudal peduncle of the male was in close contact with the left side of the female's caudal peduncle with the caudal fin of the male extending slightly beyond that of the female. The entire tail regions of both fish were bent sharply to the right. As soon as the male moved up and across the female, the force of his weight appeared to be exerted upon her, for she was pressed down into the algal mass, and simultaneously with the male's embracing movement both fish began to vibrate. The mouth of the female opened during this vibration, but the mouth of the male was not visible. About 2 seconds after this excited vibratory movement began a few beadlike objects were seen in the algal mass near the vent of the female; in the red light the sex products of the male could not be distinguished. At 8 seconds from the initiation of the vibratory movement the female moved jerkily forward about 1 inch, carrying the male with her. This movement of the female was accomplished by a frantic beating of the pectorals, as though the female were seeking to escape from the male. At the end of 13 seconds of vibration the male relaxed and fell to the algal mass among the newly-laid eggs; the female turned almost at right angles and darted to the side of the rock nearest the inflow end. Both fish remained in the positions assumed after the spawning act for about 3 minutes. During this time respiration was very rapid. After this the fish appeared less agitated, as indicated by a more normal rate of respiration.

At 2:25 a.m. the large female turned and faced the male that still occupied the central area of the rock. The male shifted slightly, conveying the impression to the observer that his weight had been lifted off the algal mass. The large male turned right and darted to the trough bottom almost directly under the incoming water supply. Within a few seconds the male turned to face the same direction as the female but about 5 inches behind her. The male then dashed

toward the female, coming to rest on the trough bottom at the inflow corner of the rock nearest the observer. The male was respiring rapidly, but the female was not. These relative positions were held for about 1 minute. The female, with a backward sweep of her pectorals, moved forward about one-half inch, and at the same time the male started a series of jerking movements which carried him up the sloping edge of rock until his head was just over the upper edge. These positions were held for about 2 minutes and both fish were respiring rapidly. The male, now about 1 inch to the left of the female's caudal fin, moved with one stroke of the tail to the left side of the female, with the tip of his snout just behind the base of her left pectoral. The long axis of the male's body was held at an angle of approximately 30° to that of the female. Rapid respiration continued in both and after about 1 minute the male raised himself above the female in the X-position, placing his pelvics directly upon her first dorsal. Upon this contact both dorsals of the female collapsed, and the female moved forward about one-half inch, the movement being effected by the tail. Most of the weight of the male appeared to be distributed upon the caudal peduncle because the female was not pressed into the algal mass to any appreciable extent. This position was held about 4 minutes and the female quivered slightly four times. The male slowly assumed the embracing position described in the first spawning act in which the downwardly bent body crossed the female's and his right side was in contact with her left for a considerable distance posteriorly. Again the tail regions of both were sharply bent to the right. When embraced, the female moved forward about one-quarter inch and down into the algal mass; vibration of both fish began with the embrace and 3 seconds later eggs could be seen pushing out from the vent of the female. Vibration lasted 8 seconds. Upon its termination the male again collapsed and fell into the newly-laid eggs. The female darted to the outflow edge of the rock. This spawning act occurred with the long axis of the female at right angles to the observer's line of sight, and during most of the vibratory period the mouth of the female was agape; once the female closed the mouth in a gasping manner.

During this spawning act the smaller male remained at his position alongside the screen partition, but the smaller female had moved to the far corner of the outflow end of the compartment. Neither of these fish displayed any interest in the activities of the older fish or in the eggs.

The larger fish that had spawned twice remained in the positions assumed after spawning for almost 30 minutes, when at 3:05 a.m. the female executed a right turn to face the center of the rock. In one dash the male approached the female on her right side. The female immediately moved about 5 inches to the far corner of the rock on the inflow edge. Both fish respired rapidly at this point. The male moved to the position just vacated by the female. After 1 minute the male approached the female in a rush with the tip of his snout behind the base of her right pectoral. The rate of respiration increased. The angle of the long axis of the male's body was less than 30 degrees to that of the female. The caudal fin of the female was bent sharply to the right, that of the male to the left. This position lasted for about 30 seconds. The male then rose above the

female to rest his pelvics upon her first dorsal. As he rose, the tail region of the female bent sharply to the left and she moved forward about one-quarter inch. Both dorsals of the female were collapsed. This mounted X-shaped position lasted about 90 seconds and then the male bent downward with his tail curved sharply to the left and embraced the female. The mouth of the female opened and vibration of both fish began; the female moved forward again about one-quarter inch, carrying the male. Three seconds after the beginning of vibration, eggs began to pour from the female's vent. At about the tenth second of vibration the female moved forward about 1 inch, still emitting eggs, and after 12 seconds of vibration the male collapsed and fell into the eggs. The female darted to the far corner at the inflow end of the compartment.

At about 3:22 a.m. the large male, now in the resting position among the newly-laid eggs, moved to the far corner of the rock, then to the trough bottom, finally coming to rest beneath the overhang of rock on the inflow edge. No further activity was observed in the compartment although observation continued until 4:45 a.m. The smaller male moved from the point near the outflow screen up the sloping edge of rock. Although he moved up on the rock and remained for about 10 minutes, he displayed no interest in the eggs. This male finally came to rest on the trough bottom.

At 4:45 a.m. the *Cladophora*-covered rock was removed and placed in a container of water from Salmon Creek. An examination of the rock showed that the eggs had been deposited so that the eggs of each spawning act could be distinguished. A total of 119 eggs had been spawned, 42 in the first act, 16 in the second, and 61 in the last. The eggs occurred singly or in clusters of up to 8, and each egg was attached to an algal filament or to another egg, or to both. During the witnessed spawning acts the water temperature was 58°F. and the water flow rate was approximately one-half gallon per minute.

During the following two days and nights both males at different times rose above the larger female to assume the X-position over her with pelvics resting upon the first dorsal. On these occasions both dorsals of the female collapsed, but neither of the fish displayed any visible excitement. This assumed position might last from 2 to 35 minutes, either in daylight or in darkness, whether the female was on the alga-covered rock or trough bottom. The weight of the male did not appear to be resting on the female during these positions because the female was not pressed to the substrate. No territory appeared to be defended by any fish in this period. Neither male paid any attention to the smaller female.

The two age-group III fish spawned on the third night following that of the first witnessed spawning acts. Prior to the first act on this night the males repeated the performance of alternately darting over the rock. They repeated the performance of breaking the surface of the water only to fall to the rock. The larger male spawned twice with the large female. Again the smaller fish remained detached from the entire proceedings. The darting of the males over the rock began at approximately 1:30 a.m.; first spawning occurred at 1:50 a.m. with a vibratory movement lasting about 10 seconds; the second spawning act occurred at 2:10 a.m. with a vibratory movement lasting 7 seconds. No further

spawning occurred and at 4:00 a.m. the rock was inspected for eggs. The first spawning act had produced 52 eggs and the second, 32 eggs.

Frequency of the spawning act.—From the witnessed spawning acts on two nights and the total number of eggs deposited by females during a spawning season a method was suggested for determining the number of spawning acts in which a female engages during a season.

During the first three witnessed acts a total of 119 eggs was deposited, an average of approximately 40 eggs. During the next two acts a total of 84 eggs was laid, an average of 42 eggs per act. Applying these averages of eggs per act to the nightly total of eggs spawned by the age-group III female (62.0 mm.) of Table 6, this fish spawned once or twice a night (67 eggs) to four or five times a night (192 eggs). The total number of eggs (1478) spawned by the female at the rate of 40 per act indicates that approximately 37 spawning acts were required for this female to spawn the ova complement. The other age-group III female of Table 6 compared favorably with the 62.0 millimeter female. It is recognized in these estimates that only a few females are involved and the spawning occurred under laboratory conditions.

No younger female was witnessed in the spawning act. The egg counts by spawning night shown in Table 6 offer suggestions, but any estimate must be considered speculative. Perhaps the younger females spawn as many eggs per act as the older females and are involved in fewer acts per season; on the other hand fewer eggs may be laid per act. About the same relationship exists between the average number of eggs spawned per night per female and the total number of eggs spawned per female per season; that is, the age-group I female spawns about one-half as many eggs per spawning night as an age-group II female, and about one-third as many as an age-group III female. The average number of eggs deposited per night as shown in Table 6 for the different age-groups does not afford a reliable measure of comparison because the variation in the number of eggs per night is so great.

Male greenside darters probably engage in the same number of spawning acts as the females. The apparent 1:1 sex ratio in the Salmon Creek population supports such a postulate as does the observation that all males apparently attain maturity and spawn at the same time as do the females.

Courtship behavior.—The darting and leaping performances of the males prior to the spawning act and the assumption of the X-shaped position by the male and female as described above (p. 175) may be considered courtship behavior. Only one display was witnessed in the stream that could be considered as such behavior. This display was the assumption of the X-shaped position observed in the laboratory, but it occurred with many individuals of both sexes taking part.

A curious display was observed at 2:00 p.m. on May 14, 1949 in a section of the stream where *Cladophora* covered the bedrock bottom. Within an area of approximately 5 feet by 4 feet, five or six males and six or eight females, all apparently of age-groups II and III, engaged in this display. The stream at this point is approximately 14 feet in width. Air temperature was 69°F. and water

temperature was 64°F. The females were distributed without any apparent pattern over the alga, all facing upstream, and a distance of several inches between any two. The different males approached the females from behind on either side. When about an inch from a female, the male stopped for periods of time varying from 15 seconds to 3 minutes. After that pause the male approached the female slowly and raised himself to the X-shaped position. During these maneuvers and the assumption of the crosswise position no excitement was evident in either partner. Little of the male's weight appeared to be resting on the female because she was not pressed into the alga. The X-position was held for periods of about 3 to 10 minutes, and on only two occasions during the observation period of one and one-half hours did a female appear to object to a male's advance. On both of these occasions (different females involved) the female darted upstream about 6 inches when the pelvic fins of the male contacted her dorsal. Whenever the X-position was terminated, the male lifted the caudal fin and glided off the female in the direction he was facing. His movements did not appear to disturb the female. After leaving a female the male often poked the snout into the algal mass, as though feeding. The females were not observed to do this. Often a newly-disengaged male immediately sought a new female, but never a female attended by a male. No pugnaciousness was observed on the part of any individual. During the observations the different females were attended by many males, but only by one at a time. Although the females did not move as much as the males, they moved sufficiently to preclude the possibility of suggesting any territorial preference.

During this display other fish of the fauna appeared in the area. These fishes were as follows: *Notropis cornutus chrysocephalus*, one large male, two large females, several juveniles; *N. rubellus*, a small school of twelve individuals; *Hypentelium nigricans*, three adults; and *Hybopsis biguttata*, one large male, two juveniles. These fish interfered in no way with the darters, and the darters did not appear to heed them.

At about 3:30 p.m. three male and five female greenside darters left the area to drop downstream about 20 feet to a crest in the riffle. Within 15 minutes all darters had disappeared from the display area. Attempts to witness further activity at the riffle crest failed because the fish could not be seen. No newly-laid eggs were found in the display area, only eggs of the eyed stage. About one hour after the last of the darters had left, a thorough search of the riffle crest area was conducted, but no newly-laid eggs were found. The rocks were returned to the stream, and the following morning a search of the same rocks yielded over 200 eggs less than 10 hours old. It is possible that these eggs resulted from the spawning activities of the same fish observed in courtship display the previous afternoon. Such behavior is the same as that observed in the laboratory.

Relation of the sexes during the spawning season.—From field and laboratory observations, apparently one female is attended by more than one male during spawning activities, but only by one male at a time. Similarly, a male attends more than one female during the breeding season. Promiscuity appears to be

the rule in both sexes. It was further demonstrated in the laboratory that females of any age-group could spawn successfully with males of any age-group. When pairs of fish of the same age-group were placed together in the rearing troughs, the females always spawned the full complement of ova. The same result occurred when older males were placed with younger females, but when old females (age-group III) were placed with young males spawning for the first time, the full ova complement was not deposited even though the experiment was continued past the end of the wild breeding season to July 15. This experiment was repeated the following year, except that after an age-group III female spawned approximately 600 eggs with a young male and had apparently stopped, the female was introduced to an age-group III male in breeding color. This particular female then spawned the remainder of her eggs within ten days. Two control age-group III females paired with first year males failed to spawn all their eggs although the experiment was continued until July 15. Examination of the gonads of these young males after the experiment showed the testes to be but slender threadlike processes, as though the organs were completely spent. It is indicated that first year males cannot stimulate those old females spawning for the third season to complete ova deposition.

Post-spawning season activity.—After the breeding season is over, the most striking change in the greenside darter is the loss of most of the brilliant coloration in both sexes of all age-groups and assumption of the drab coloration noted by Jordan and Copeland (1876*b:* 340) and described above (pp. 142–143,). Both sexes apparently spend most of the time in feeding, as indicated by the figures of Table 2. It is during the summer months that greater growth is indicated by scale studies in which the circuli are more widely spaced (Fig. 10). It is strongly indicated that most of those darters that survive to spawn for the third season die during the following summer.

EGGS AND OFFSPRING

Materials and methods for development studies.—Because it was impossible to strip eggs successfully from ripe females, it was necessary to obtain eggs from spawning fish in the rearing troughs of the laboratory. Eggs were picked from algal filaments by means of forceps and placed in one-half-pint, spring top, Mason fruit jars containing stream water from Salmon Creek. The chlorinated water supply, though suitable for adult fish when properly aerated, was toxic to the developing embryos. The jars were placed in rearing troughs. Water flowing through the trough at a level just below the lip of the jars served as a temperature bath. The jars were not sealed with the conventional jar-rubber, but were closed by means of the glass top and spring fastener only. The stream water in the jars needed to be changed only every two or three days. The eggs are demersal and care must be taken that no more eggs are placed in a single jar than cover the bottom area. Such simple practices reduced the total egg mortality rate from about 95 percent to approximately 20 percent.

As soon as the embryo breaks through the egg capsule, the prolarva may be transferred to an aquarium. A one- or two-gallon glass aquarium was found to

be most satisfactory from the standpoints of visibility and facility in cleaning. The aquarium may be kept in a room at room temperature. When the yolk sac is absorbed, the postlarva must be fed. Fish food preparations of the conventional commercial dry type were not ingested, neither were finely-ground beef liver nor particles of chicken egg yolk. Plankton obtained by towing a No. 12 plankton net through the deeper riffle sections of Salmon Creek was found to be ingested by the postlarva.

Eggs and larvae were removed from the containers by means of wide-mouthed pipettes and were placed in watch glasses or depression slides to be observed. During such microscopic examinations the use of a heat filter is essential. Embryos excised from the egg capsule were placed in a double concentration of Holtfreter's standard solution (Rugh, 1948: 395). Holding the encapsulated embryo between the points of No. 5 watchmaker's forceps, the capsule was punctured with the point of a Bard and Parker No. 11 scalpel. In this operation the embryo must be positioned in such a manner that the puncture can be made at a point not in contact with the large yolk mass. Consistently good results were obtained at a point immediately dorsal and lateral to the posterior region of the head. Here there is an area between the chorion, head, and the plasma membrane of the yolk sac.

Free-swimming prolarvae and postlarvae were drawn with camera lucida while narcotized in a 0.5 percent urethane(Merck)-Salmon Creek water (filtered) solution. All larvae survived narcotization at the above concentration for periods up to 4 minutes. Recovery required 1 to 5 minutes. A postlarva narcotized for 8 minutes required 3 hours to recover. This particular postlarva appeared normal at that time, but approximately 8 hours after removal from the urethane it died.

Description of the egg.—The fertilized egg of *blennioides* is spherical, transparent, demersal, and adhesive. A prominent yellow oil globule is present. Eggs are laid singly or in clusters of up to thirteen. At the place of adherence the egg capsule is flattened. Frequently the adhesive covering appears to develop a fine reticulate surface sculpture. The average diameter of an egg is 1.85 mm. (range = 1.75 to 1.98 mm.). Ten minutes after fertilization the egg is transparent and water-hardened. The cytoplasm is clear, and suspended in it is the fluid, colorless yolk mass of average diameter 1.43 mm. (range = 1.33 to 1.47 mm.); within the plasma membrane of the yolk mass is found the single, yellow oil droplet of 0.63 mm. diameter, and surmounting the yolk mass is the disc of protoplasm that ultimately gives rise to the embryo.

Unfertilized eggs differ from fertilized ones in being translucent, cuboidal, non-adhesive, and not water-hardened. Unfertilized eggs were seldom seen in the rearing troughs and never in the stream. Being non-adhesive, such eggs were presumably carried away in the stream.

Cleavage stages.—The development of the egg through the cleavage stages is given as observed in the laboratory with water temperatures varying from 55° to 58°F. Fertilization time is taken as that moment when both parents are vibrating during the spawning act and eggs are visible as they are extruded from the female.

The first cleavage depression bisecting the blastodisc is manifest within 55 to 60 minutes after fertilization. The first cleavage is completed within 65 minutes after fertilization (Fig. 5, A). The axis of the second cleavage depression passes through the center of the blastodisc at right angles to the first cleavage plane within 25 to 30 minutes after the appearance of the first cleavage depression, and 3 to 5 minutes are required to complete the formation of the 4-blastomere stage (Fig. 5, B). After this a new cleavage is initiated every 28 to 30 minutes. Each blastomere of the 4-blastomere stage cleaves in a plane parallel to that of the first cleavage to form an 8-blastomere plate, all of the blastomeres equal in size (Fig. 5, C). Each blastomere of the 8-blastomere stage cleaves along a plane parallel to that of the second cleavage giving rise to a 16-blastomere plate, all of the blastomeres about equal in size (Fig. 5, D). As with most teleost fishes, the fifth cleavage is equatorial and divides the 16-blastomere plate into an upper and a lower component (Fig. 5, E). In this stage the 32 blastomeres are about equal in size. After this stage it is difficult to decide whether or not the cleavage of the individual blastomeres is synchronized according to the methods employed (observation of living eggs under direct light using a dissection microscope at ×12.5 to ×60.0 magnifications). Apparently a cleavage is initiated approximately every 30 minutes up to 6 hours after fertilization. The blastodisc now appears granular at a magnification of ×28.75 and is in an elevated position surmounting the yolk mass. Eight hours after fertilization the beginning of epiboly can be detected. This downward movement of cells is well under way 12 hours after fertilization, and the blastoderm covers approximately one-third the yolk mass (Fig. 5, F), about one-half at 24 hours, and one-half to three-quarters at 37 hours. The oil droplet is contained in the region of the yolk plug during epiboly.

Formation of the embryo.—Thirty-eight hours after fertilization the peripheral region of the blastoderm presents a thickened appearance, and the germ ring can be recognized. The upper portion of the blastoderm becomes less dense. The embryonic shield is visible as shown in lateral view in Fig. 5, G. The rudiment of the embryo forms over the circumference of the yolk mass, and at 57 hours the head, trunk, and tail regions are recognizable. The optic vesicles are clearly visible, and forebrain and midbrain regions are indicated. The trunk and tail regions of the embryo are flat and cling closely to the plasma membrane of the yolk mass for about one-half its circumference (Fig. 5, H). Melanophores first appear upon the yolk mass at about 64 hours, and the body of the embryo becomes thicker and more rounded in the posterior region. Mesodermal somites first appear in the trunk region at about 72 hours. The lens placode is apparent within its optic vesicle at 72 hours. The head, trunk, and tail are still more rounded and raised higher upon the yolk mass; mesodermal somites are now visible throughout the trunk region; melanophores are more numerous upon the yolk sac and are also evident on the ventral surface of the tail region, both in dendritic and punctulate form; the extreme tip of the tail is rounded and free from the yolk sac, and occasionally movement in the tail-tip is observed; the entire length of the embryo at this time extends about half-way around the yolk sac (Fig. 5, I).

The tail region is completely free from the yolk mass and moves from side to

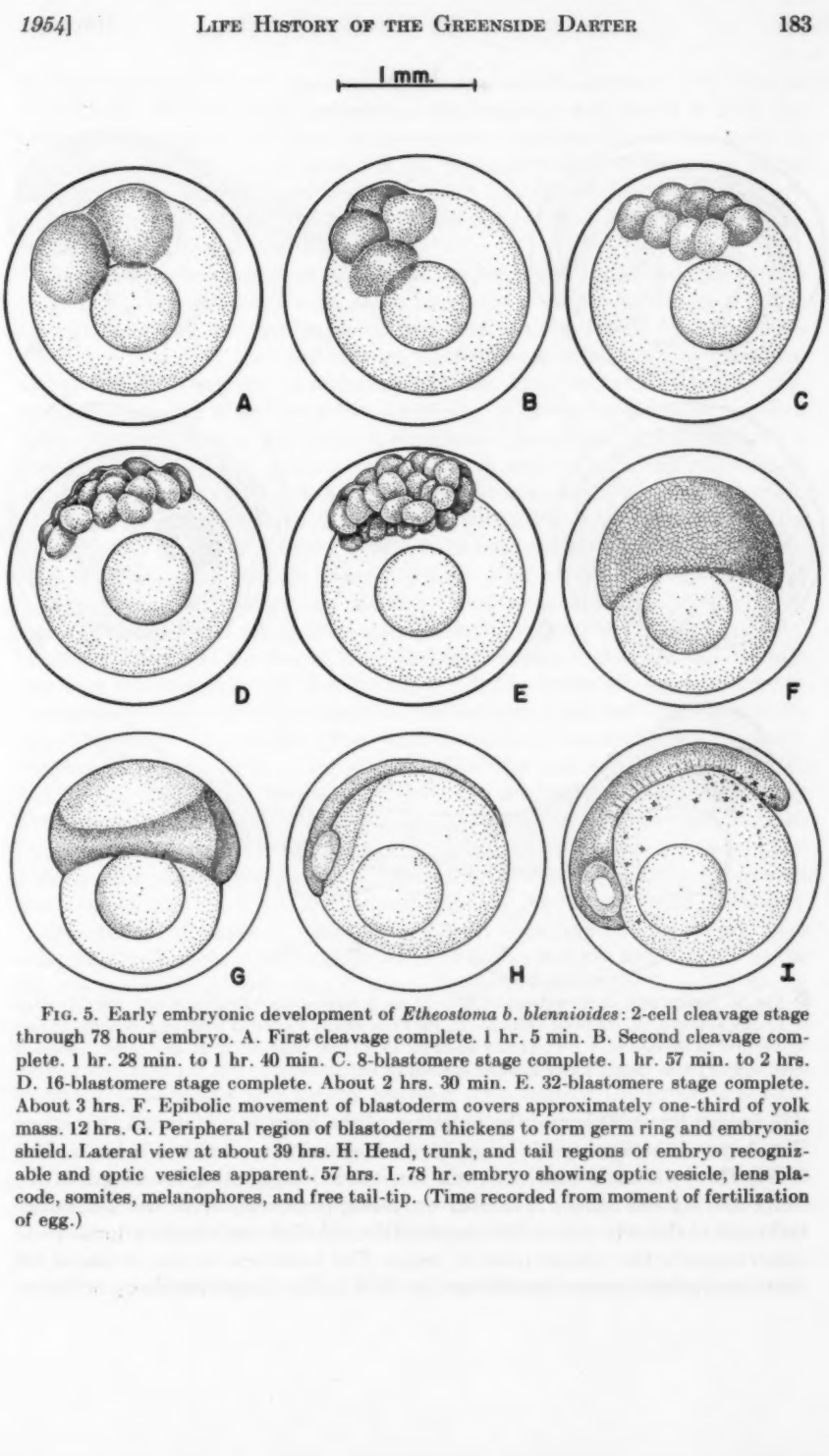

FIG. 5. Early embryonic development of *Etheostoma b. blennioides*: 2-cell cleavage stage through 78 hour embryo. A. First cleavage complete. 1 hr. 5 min. B. Second cleavage complete. 1 hr. 28 min. to 1 hr. 40 min. C. 8-blastomere stage complete. 1 hr. 57 min. to 2 hrs. D. 16-blastomere stage complete. About 2 hrs. 30 min. E. 32-blastomere stage complete. About 3 hrs. F. Epibolic movement of blastoderm covers approximately one-third of yolk mass. 12 hrs. G. Peripheral region of blastoderm thickens to form germ ring and embryonic shield. Lateral view at about 39 hrs. H. Head, trunk, and tail regions of embryo recognizable and optic vesicles apparent. 57 hrs. I. 78 hr. embryo showing optic vesicle, lens placode, somites, melanophores, and free tail-tip. (Time recorded from moment of fertilization of egg.)

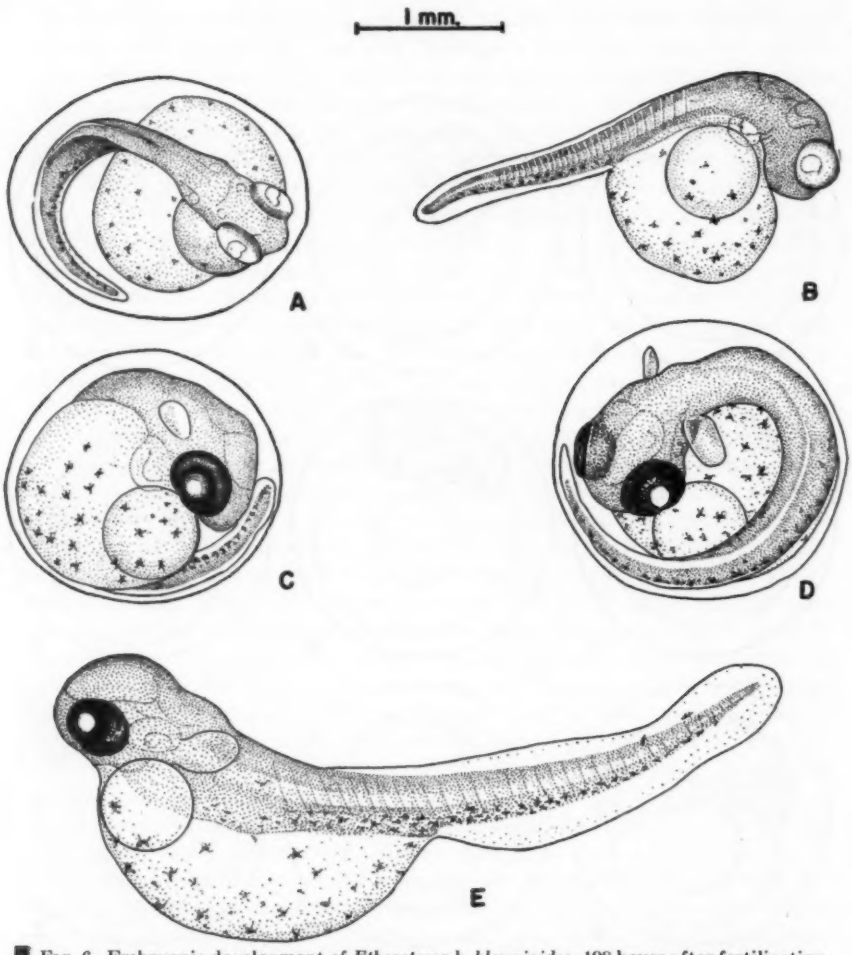

FIG. 6. Embryonic development of *Etheostoma b. blennioides*: 108 hours after fertilization to 13 day embryo. A. 108 hour embryo. B. 108 hour embryo excised from egg capsule. (Same individual as figured in A.). C. 11 day embryo. D. 13 day embryo. E. 11 day embryo excised from egg capsule (Same individual as figured in C.).

side frequently at 90 hours; the pulsation of the heart is initiated in this stage. The shape of the egg capsule is not so much spherical as oval at 108 hours (Fig. 6, A). The yolk sac is smaller at this time, thus exaggerating the length of the freely-moving tail region. A fin-fold extending posteriorly from the ventral attachment of the yolk sac continues around the tail of the embryo to a dorsal point about opposite the ventral point of origin. The heart beat is now strong at 134 beats per minute (water temperature = 58°F.). The dorsal periphery of the re-

tina is beginning to show pigmentation; the vacuolated notochord tissue extends far back into the tail region; mesodermal somites number about 27 or 28 at this period; melanophores are more numerous than previously upon the yolk sac membranes and the ventral surface of the posterior portion of the trunk region and tail; differentiation of the brain continues, and the otic capsules are in evidence in the head region. The length of the embryo from head to tail extends about three-quarters around the yolk sac.

To facilitate observation, an embryo of 108 hours was excised from its capsule (Fig. 6, B). Measurements were made by means of an ocular micrometer. An approximate total embryo length of 2.83 mm. was obtained. Because of the downward flexure of the head only an approximate total length measurement could be made. The yolk sac measured 1.13 mm. along its antero-posterior axis, and the diameter of the oil droplet was 0.58 mm. At one hundred and twenty-four hours after fertilization the retina is completely pigmented, and the embryo is very active, rotating vigorously within the capsule at intervals. Circulation through the dorsal aorta and vitelline vessels is apparent. The blood color is a very light pink. Total length of the embryo within the capsule is such that the tip of the tail meets the anterior point of the head. Between 120 and 150 hours after fertilization the capsule apparently loses most of its adhesive qualities so that pieces of algal filaments and particles of sand that cling to its surface can be removed easily. From the seventh through the eleventh day the embryo's total length increases and the size of the yolk sac decreases so that the tail overlaps the head region within the capsule (Fig. 6, C). The melanophores are more numerous on the yolk sac and ventrally on the trunk and tail, but no melanophores appear dorsally. Laterally along the somite boundaries melanophores are present. Somites number 37 to 38 at the eleventh day. The pectoral fins appear at the eighth day, and at the eleventh day these fins are fan-shaped with no fin-ray elements indicated (Fig. 6, C, E). The gut is evident as a straight tube running from the region of the oesophagus to the ventral attachment of the fin-fold and yolk sac; the gut is not open to the exterior. An eleven-day embryo was excised from the capsule (Fig. 6, E). At this stage the head is no longer flexed downward. Differentiation of the skull continues and both brain and otic region are more complex. The fin-fold is still continuous, but both ventrally and dorsally in the tail region this fold is beginning to notch, delimiting the ultimate caudal fin; no fin-ray element is indicated anywhere in the fold. The total length of the embryo is 4.77 mm.; the relative decrease in size of the yolk sac is most apparent in a dorso-ventral plane rather than in an antero-posterior one. The oil droplet is now 0.52 mm. in diameter and almost colorless. The embryo has attained a total length of 5.33 mm. at 13 days. The pectorals lose the roundness of outline and become more paddle-shaped, as shown in Fig. 6, D. The embryo is very active and rotates frequently within the capsule. The yolk sac decreases to a point that enables the tail to coil around the trunk and yolk sac twice at 16 days. The lower jaw is almost completely formed at this stage. The embryo continues to rotate frequently.

Hatching.—Eighteen days after fertilization, with water temperatures between

55° and 58°F., hatching begins. For a particular batch of eggs the hatching period extends over a period as long as 48 hours. Of 41 observed hatchings 39 embryos emerged tail-first, and the other two hatched head-first. Of these latter, one was visibly abnormal, sharply contorted and only 4.60 mm. in total length; this specimen died 10 minutes after hatching. The other appeared to be normal. It is indicated that tail-first emergence is the rule in the species.

For 24 hours prior to hatching, the tail of the embryo lashes almost constantly. After breaking through the capsule, the prolarva (Hubbs, 1943: 260) struggles to free itself from the clinging capsule that still encloses the head, pectoral fins, and yolk sac. Frequently, hatched individuals were discovered that had died before ridding themselves of the capsule; in all these fatalities more than 5 hours had elapsed since the moment of hatching. When embryos were experiencing difficulty in freeing themselves from the hindering membrane, forceps were carefully used to tear the capsule. Embryos so aided gave no evidence of being harmed.

Embryos emerging on the eighteenth day after hatching encounter greater difficulty in freeing themselves from the ruptured membrane than those hatching on the twentieth day, presumably because of the larger size of the yolk sac. Twentieth-day embryos free themselves in periods varying from 20 minutes to about 1 hour and 35 minutes, but 18-day embryos require as long as 5 hours. These times may be atypical, however, because in the laboratory the struggling embryos are in a glass container in quiet water, but in the stream the embryos would be in swiftly running water. Perhaps the embryos can utilize the movement of water through the algal mass to free themselves.

After the tail of the embryo breaks through the egg capsule, the prolarva lies still upon the bottom of the dish, the venter in contact with the glass. The pectorals, still within the capsule, continue to beat. This relatively quiescent period, lasting 2 to 15 minutes, is followed by a period of intermittent tail lashing and general thrashing of the body until the capsule is cast off.

Prolarva.—The newly-hatched prolarva varies from 6.75 to 7.50 mm. in total length. Twenty-four hours after hatching, the melanophores are dendritic upon the yolk sac and peritoneal covering of the gut, but on the ventral surface of the body along the myomeres the shape of the melanophores tends to be punctulate; no melanophores are apparent on the dorsum at this stage (Fig. 7, A, B, C). Among the fins only the pectorals show fin-ray elements. The fin-fold is notched deeply at this time at the point where the digestive tube seeks an exit to the exterior. The head region is well differentiated. The mouth, though weakly formed, is open, and water passes through the mouth, over the gills and out the gill slits. Movement of the operculum is visible. Within the yolk sac the oil droplet is visible, but colorless. The heart beat is evident and circulation throughout the body can be seen.

When water temperature is 60°F., the prolarva requires 6 days to absorb the yolk sac. During the third or fourth day of prolarval life very few melanophores are apparent along the myomeres of the dorsum of the tail region. The vent opens to the outside at this time. Later, bits of green-brown material are observed

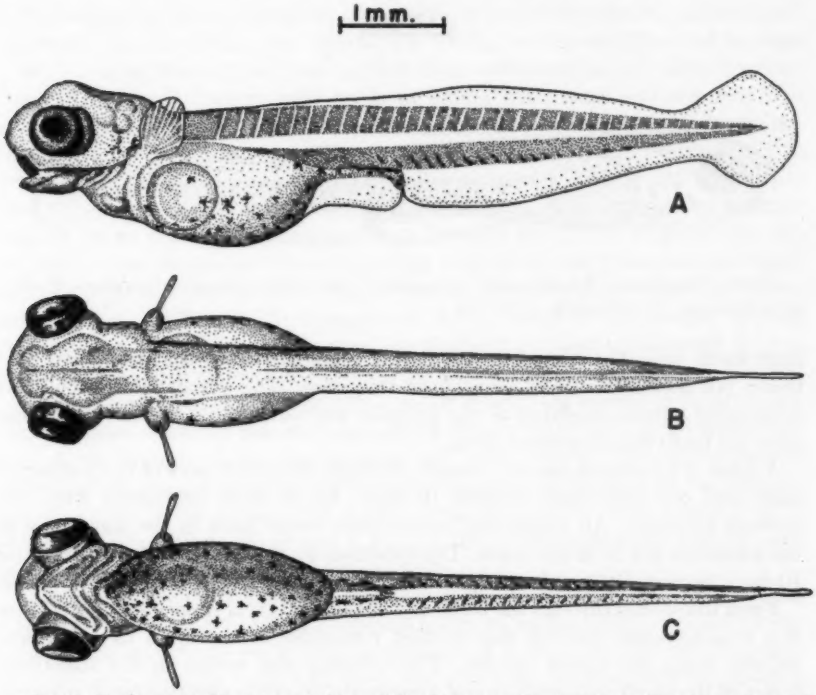

FIG. 7. Prolarva of *Etheostoma b. blennioides* 24 hours after hatching. Total length = 7.5 mm. A. Lateral View. B. Dorsal View. C. Ventral view.

hanging from the vent, presumably undigested material from the yolk mass. As the yolk disappears so does the oil droplet. From the fourth through the sixth day of prolarval life the mouth becomes larger and more terminal in position. On the fifth and sixth days melanophores appear upon the cheeks and opercles.

Throughout the prolarval period the animal is active and can be found at all levels of the aquarium. If a mass of green alga is available, a considerable part of the time is spent upon, or within, the tangled mass of filaments. When searching for such specimens the large pigmented portion of the eye is most conspicuous as a recognition mark. This stage was never observed in the wild.

Postlarva.—For approximately 24 hours after the disappearance of the yolk sac the fish does not feed, but on the second day of the postlarval period active feeding has been observed. Ten days after yolk sac absorption the postlarva appears as shown in Fig. 8. The pectoral fins are prominent with well developed fin-rays. The caudal is quite sharply defined and fin-rays are apparent. At the base of the fin-fold in the regions of the second dorsal, anal, and pelvic fins, fin-ray elements can be discerned. Melanophores occur ventrally and dorsally, especially along the myomeres. The head has changed greatly from the prolarval stage. It is characterized by a large terminal mouth with numerous, relatively

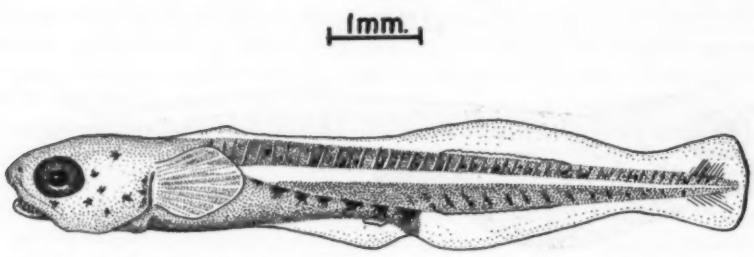

FIG. 8. Postlarva of *Etheostoma b. blennioides* 10 days after yolk sac absorption, 16 days after hatching. Total length = 8.0 mm.

large teeth upon the dentaries, and darkly pigmented eyes that no longer protrude. No postlarva was observed past this stage. The overhanging snout and subinferior mouth condition of the juvenile and adult must appear some time after the tenth day of postlarval life.

A total of 21 individuals was brought through the first 8 days of the postlarval stage and one individual reached 10 days. All of these postlarvae were lost through accidents. An undetected carnivorous beetle larva in the algal mass of the aquarium ate 20 in one night. The specimen shown in Fig. 8 succumbed after 10 days, apparently overdosed by narcotizing agent during the drawing process.

From the second through the tenth day of the postlarval stage a striking feature is a brown-orange coloring that is most noticeable in the brain case and posteriorly along the spinal column. This coloring also occurs in the superficial layers of the trunk musculature and apparently upon the peritoneum. It does not appear to be within chromatophores, but resembles a fluid. Its intensity has decreased considerably by the tenth day.

Earliest young-of-the-year fish collected on August 8 (1948) varied in total length from 30.0 to 37.0 mm. The postlarva of Fig. 8 had reached a total length of 8.0 mm. on June 20 (16 days after hatching). To attain the total length of the earliest young-of-the-year such a postlarva must grow 22.0 mm. or more in a period of approximately 48 days. It is indicated that the time of transformation of the postlarva to the juvenile stage occurs in very late June and July. These data, though comparing larvae in the laboratory with wild specimens in a different year, provide the best estimate of transformation time that is available.

AGE AND GROWTH STUDIES

Determination of age by the scale method.—Age determinations based upon the scale method have been used successfully in the growth studies of percid fishes such as *Stizostedion vitreum* (Adamstone, 1922; Deason, 1933; Hart, 1928) and *Perca flavescens* (Harkness, 1922; Hile, 1931; Hile and Jobes, 1940; Jobes, 1933; Schneberger, 1935, and Webster, 1942). Raney and Lachner (1943) were the first to apply the scale method to darter growth studies when they investigated *Etheostoma nigrum olmstedi* and *E. longimanum*. Lachner *et al.* (1950) studied age and growth of the darters *E. b. blennioides*, *E. variatum*, and *E. z. zonale*; this study was unknown to me during my investigations.

Preliminary observations of the scales of *E. b. blennioides* showed a differential spacing of the circuli and also indicated a cutting over mark described by Hile (1931). During the calendar year of 1948 in the first week of each month collections of greenside darters were made including both sexes and different size groups. The primary purpose of these collections was to follow the development of the scale of both sexes and different size groups throughout the year and to determine the time of formation of the cutting over mark. This monthly examination showed that spacing between the circuli was wider during the warmer months (May through September) than through the colder months. By this method it was established that the cutting over mark was formed in late April or early May and only at that time in both sexes and in all size groups. Raney and Lachner (1943: 230–33) encountered the condition of differential spacing between circuli in *E. nigrum olmstedi*, but they did not find cutting over in that species. These authors recognized the wider spacing of the circuli as representing periods of more rapid summer growth, and conversely, the regions of more closely approximated circuli as representing periods of slowing growth or growth cessation. As an annulus, Raney and Lachner (*loc. cit.*) specified the point in the anterior field of the scale between the cessation of growth in the fall and the resumption of growth in the spring. Such a point is clearly evident in the scales of *E. b. blennioides* (Fig. 10) in age-groups I, II, and III; in the same scales cutting over marks are evident in one or both lateral fields.

When scales are taken from the conventional body site, that is, from the right side above the lateral line in the vertical scale row immediately anterior to the origin of the first dorsal fin, regeneration is found to occur frequently. To determine the most suitable body area from which to take scale samples for possible age determinations 10 darters collected on the same day were used. Among these 10 were 5 males measuring 39.0, 47.5, 57.5, 63.0, and 72 mm. in standard length and 5 females measuring 33.5, 43.5, 52.0, 62.0, and 71.0 mm. in standard length. From each fish a sample of four scales was taken from each of seven body areas located on the right side of the fish. The first body area was the conventional one described above. The second body area was located just below the lateral line in the two vertical scale rows immediately posterior to an imaginary line drawn vertically from the origin of the first dorsal fin. Two areas were located on the caudal peduncle, one above and one below the lateral line. Two more areas were located above and below the lateral line at mid-body. A seventh area was adjacent to the mid-line of the venter anterior to the anus.

The scales were cleaned and mounted permanently in Sayre's medium (Sayre, 1941: 160) modified after Uphoff (1948: 62). The microprojector used was that described by U. Stone (1947: 239-43). Comparison was made among the scales from different areas on the basis of size, percent frequency of regeneration, and condition of circuli. In all characteristics the scales from the second body area described above were most satisfactory, and scales from this area were used in the age determination study. When scales from this second area were unsatisfactory in any specimen, scales were taken from the conventional site.

With knowledge of time of year of formation of the cutting over mark and the recognition of the presence of differential spacing between circuli, an attempt was

TABLE 7

Length-frequency distribution in three-millimeter class intervals of 126 Etheostoma b. blennioides *taken January 4, 1950 from Salmon Creek, Monroe County, New York*

S. L. in mm.	Males	Females
33.0–35.5	—	1
36.0–38.5	—	3
39.0–41.5	5	8
42.0–44.5	6	2
45.0–47.5	4	—
48.0–50.5	—	—
51.0–53.5	2	1
54.0–56.5	3	4
57.0–59.5	13	16
60.0–62.5	7	7
63.0–65.5	9	6
66.0–68.5	12	4
69.0–71.5	5	1
72.0–74.5	5	—
75.0–77.5	2	—
Total	73	53

made to establish the validity of (1) the point on the anterior field of the scale between regions of closely approximated circuli and widely spaced circuli, and (2) the cutting over mark in the lateral field, as true year marks or annuli. A collection of 126 darters taken on January 4, 1950, the largest single day's sample available, is given as a length-frequency distribution at 3-millimeter class intervals in Table 7. In Fig. 9 the standard lengths of the sexes (73 males, 53 females) are plotted against their frequency based upon Table 7. The graph for the males in Fig. 9 shows two size groups, the smaller extending from 39.0 to 47.5 mm., and the larger from 51.0 to 77.5 mm. It is indicated that more than one age group is present in the larger size group because of the presence of two modes. The graph for the females showed two size groups, the smaller extending from 33.0 to 44.5 mm., and the larger from 51.0 to 71.5 mm.

The suggestions of Hubbs (1943: 260) regarding the designation of age-groups have not been followed in this instance. To avoid confusion, the specimens collected on January 4, 1950 have been treated as though collected prior to January 1, thus age-group O fish had passed through one growing season, age-group I fish through two growing seasons, and so forth.

A scale from one of the larger males (47.5 mm.) and one of the larger females (43.5 mm.) in the smaller size groups of Fig. 9 are shown in Fig. 10, O. There is no indication of cutting over in either. These scales are typical for all those specimens contained in the smaller size group of each sex in Fig. 9. From a male (57.5 mm.) and a female (52.0 mm.) contained in the first part of the larger size group of their respective graphs in Fig. 9, scales are shown in Fig. 10, I. In both of these scales, at a point about midway between the focus and the periphery of

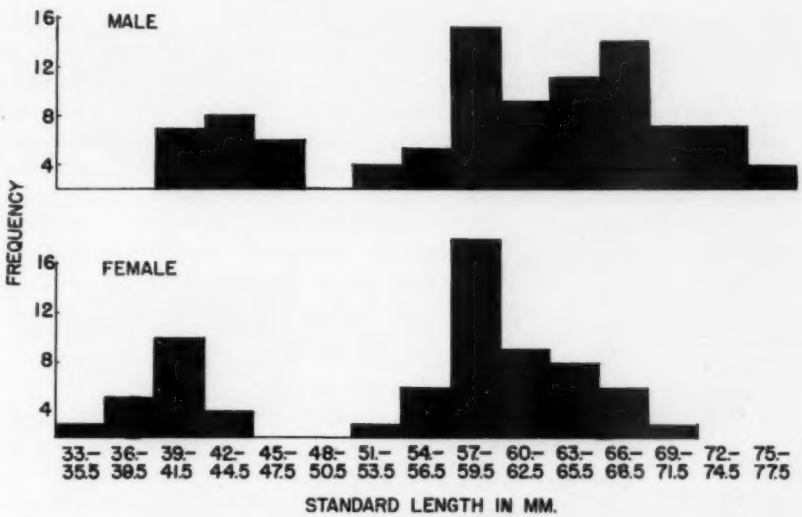

FIG. 9. Length-frequency distribution in 3-millimeter class intervals of the standard lengths of 73 male and 53 female *Etheostoma b. blennioides* collected January 4, 1950 in Salmon Creek, Monroe County, New York, based upon the figures of Table 7.

the lateral fields, there occurs a cutting over mark. If that mark is traced forward into the anterior field, it is seen to be the dividing line between a posterior region of closely approximated circuli and an anterior region of more widely spaced circuli. As these widely spaced circuli approach the anterior periphery, they become more closely approximated in both scales. In the anterior fields of each of these two scales then are found two areas, each area comprised of a region of widely spaced circuli followed by a region of closely approximated circuli, and the two areas are divided clearly by a line that can be recognized as a cutting over mark when traced into the lateral fields. From a male (63.0 mm.) and a female (62.0 mm.) contained in approximately the mid-portion of the larger size group of their respective graphs in Fig. 9, scales are shown in Fig. 10, II. In these scales two cutting over marks are apparent in the lateral fields, the first approximately midway between the focus and scale periphery and the second approximately two-thirds of the distance between the focus and the scale periphery. Traced anteriorly, these cutting over marks can be seen to delimit three areas of differentially spaced circuli. (At the magnification of 25 diameters shown in the photomicrographs of Fig. 10 this differential spacing is not as pronounced as it would be with the increased magnification of the microprojector.) From a male (72.0 mm.) and a female (71.0 mm.) contained in approximately the last part of the larger size group of their respective graphs in Fig. 9, scales are shown in Fig. 10, III. In each of these scales three cutting over marks are evident and in the anterior field these cutting over marks delimit four areas of differentially spaced circuli.

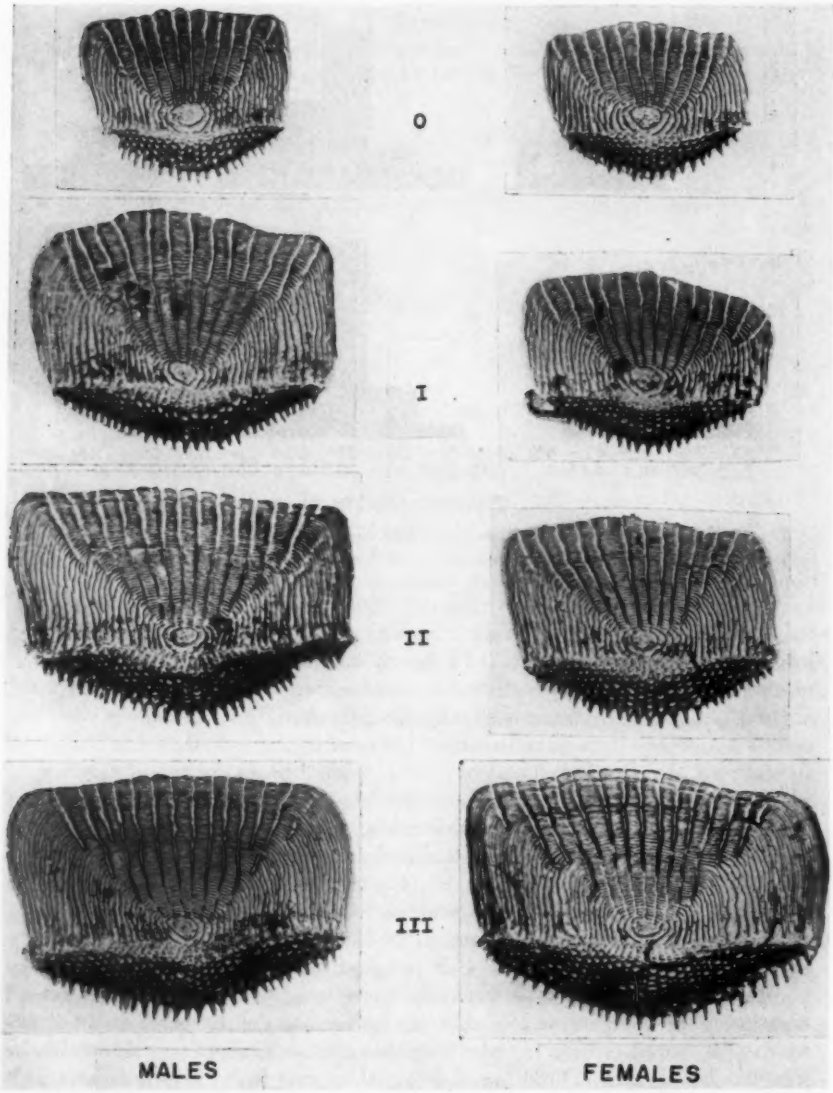

FIG. 10. Photomicrographs of representative scales of *E. b. blennioides* of both sexes in four different age-groups taken from the area immediately below the lateral line in the two vertical scale rows posterior to an imaginary line drawn vertically from the origin of the first dorsal fin. Scales are from fish collected January 4, 1950 from Salmon Creek, Monroe Co., New York. Standard lengths of the males for the different age-groups: 0, 47.5 mm.; I, 57.5 mm.; II, 63.0 mm.; III, 72.0 mm. Standard length of females for the different age-groups: 0, 43.5 mm.; I, 52.0 mm.; II, 62.0 mm.; III, 71.0 mm. Magnification ×25.

Thus, typical scales of *Etheostoma b. blennioides* of both sexes collected on the same day show an increasing number of areas of differentially spaced circuli and cutting over marks with progressively larger fish. The monthly scale studies show that circuli are more widely spaced during warm months of May through September and more closely spaced during colder months. The same study demonstrates that only in late April or early May is a cutting over mark formed, and if traced into the anterior field of a scale the cutting over mark actually delimits the period of growth cessation posteriorly and the period of growth resumption anteriorly. Therefore, an area on the scale comprised of a region of widely spaced circuli and a region of more closely approximated circuli with the delimiting cutting over mark represents one year of time in the life of the fish.

The 126 greenside darters collected on January 4, 1950 were aged by the scale method and in Table 8 a length-frequency distribution according to age and sex for this collection is shown. In Fig. 11 the results of this breakdown are shown graphically. The graph for the males presents two size groups as in Fig. 9. The first size group is composed entirely of age-group O individuals, the second of four different age-groups. In the second size group, one age-group O specimen is contained in the 51.0–53.5 mm. interval; the age-group I individuals are found in the size range of 51.0 to 68.5 mm.; the age-group II specimens are contained in the range of 60.0 to 77.5 mm.; one age-group III fish occurred in the 72.0–74.5 mm. interval. The graph for the females presents two size groups. The first size group is composed entirely age-group O fish, the second of three different age-

TABLE 8

Length-frequency distribution according to age and sex of 126 Etheostoma b. blennioides *collected from Salmon Creek, Monroe County, New York, on January 4, 1950*

S.L. in mm.	Age-Group								Totals for all age-groups		
	O		I		II		III				
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂ + ♀
33.0–35.5		1							—	1	1
36.0–38.5		3							—	3	3
39.0–41.5	5	8							5	8	13
42.0–44.5	6	2							6	2	8
45.0–47.5	4								4	—	4
48.0–50.5	—								—	—	—
51.0–53.5	1		1	1					2	1	3
54.0–56.5			3	3		1			3	4	7
57.0–59.5			13	6		10			13	16	29
60.0–62.5			6		1	7			7	7	14
63.0–65.5			5		4	5		1	9	6	15
66.0–68.5			1		11	4		—	12	4	16
69.0–71.5					5			1	5	1	6
72.0–74.5					4		1		5	—	5
75.0–77.5					2				2	—	2
Number of specimens.....	16	14	29	10	27	27	1	2	73	53	126

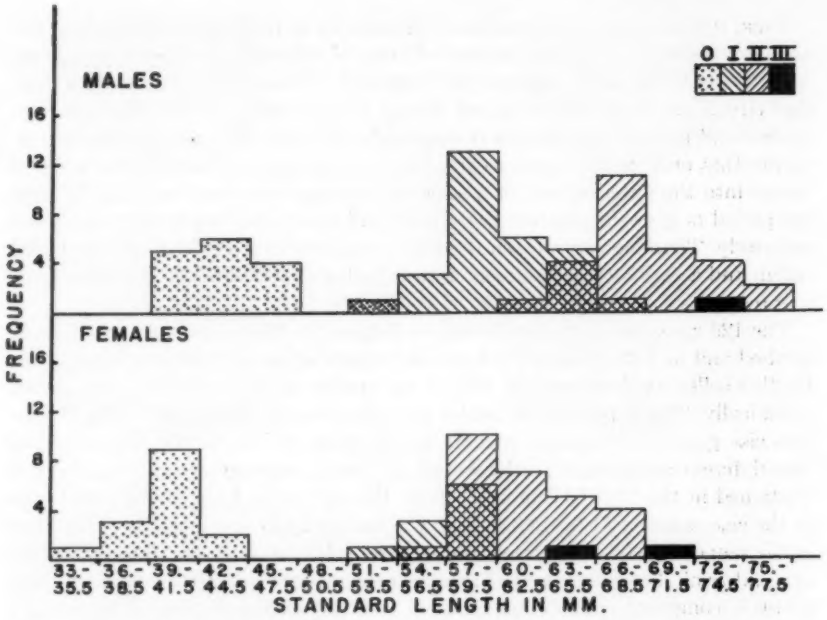

FIG. 11. Length-frequency distribution according to age and sex of 73 male and 53 female *E. b. blennioides* collected January 4, 1950 in Salmon Creek, Monroe County, N. Y. Graphs based upon the figures of Table 8.

groups. In the second size group age-group I specimens are contained in the intervals 51.0 to 59.5 mm., age-group II fish in the 54.0 to 68.5 mm. intervals, and age-group III fish in the 63.0 to 65.5 mm. and 69.0–71.5 mm. intervals. There is a close agreement between the distributions of Fig. 9 and Fig. 11, but, as pointed out by Raney and Lachner (1943: 234–35), inaccuracies may result when attempting to estimate the age of fish from length-frequency distributions of collections. Fig. 11 clearly shows the fact that more than one age-group can be contained in one mode. On the other hand Raney and Lachner (*loc. cit.*) suggested that at least the age-group O specimens of a collection can usually be separated from the older fish. The data shown in Fig. 11 clearly support that suggestion.

On the basis of monthly scale development studies through a calendar year and the agreement of features of typical scales with the length-frequency distribution of a large collection of darters made on the same day, the cutting over mark in the lateral fields of a scale manifest anteriorly as a point of division between posterior closely approximated circuli and anterior widely spaced circuli, is established as a valid year mark, or annulus, in the population of greenside darters studied.

Growth study.—A sample of 30 or 40 specimens of *blennioides* collected during

TABLE 9

Partial summary of calculations from data afforded by a collection of 296 Etheostoma b. blennioides *from Salmon Creek, Monroe County, New York*

Collection group 1 fish taken from October 1, 1949 through November 30, 1949, group 2 taken December 29, 1949 and January 4, 1950. N = number in sample; $\bar{x}$ = mean; s = standard deviation; $s_{\bar{x}}$ = standard error of the mean.

Coll. Group	Statistic	Age-Group									
		O		I		II		III		IV	
		M	F	M	F	M	F	M	F	M	F
1	N	26	29	19	13	22	36	3	1	0	1
	$\bar{x}$	46.4	41.7	61.8	55.5	68.8	61.5	72.7	71.0	—	71.5
	s	3.08	2.44	5.07	2.61	3.89	2.68	—	—	—	—
	$s_{\bar{x}}$	0.60	0.45	1.16	0.72	0.83	0.45	—	—	—	—
2	N	17	18	33	12	28	33	1	4	0	0
	$\bar{x}$	44.6	40.4	60.0	56.0	68.5	61.4	72.0	67.0	—	—
	s	3.95	2.76	3.12	2.41	3.74	3.69	—	—	—	—
	$s_{\bar{x}}$	0.87	0.65	0.54	0.70	0.71	0.64	—	—	—	—
1 + 2	N	43	47	52	25	50	69	4	5	0	1
	$\bar{x}$	45.7	41.2	60.7	55.8	68.7	61.4	72.5	67.8	—	71.5
	s	3.38	2.62	4.00	2.48	3.77	3.18	—	—	—	—
	$s_{\bar{x}}$	0.52	0.38	0.56	0.50	0.53	0.38	—	—	—	—

three or four hours of intensive seining in Salmon Creek is an exceptionally good catch. Most samples are much smaller. Such small samples cannot be considered suitable for statistical analysis. Because of press of duties the number of collecting trips was limited, and therefore a considerable amount of time was necessary to amass a sufficient number of specimens for statistical treatment. Scale analysis showed that circuli became more closely approximated in October, the period of growth retardation; later in the winter this interspacing became still smaller and for all practical purposes growth appeared to cease. If little or no growth occurs in autumn or winter, then perhaps specimens collected at different times within that period are comparable in size, as determined by standard length. During the fall of 1949 and winter of 1949–50 collections were made as follows: 5 (Oct. 1), 22 (Oct. 8), 38 (Oct. 15), 2 (Oct. 22), 32 (Nov. 3), 24 (Nov. 12), 17 (Nov. 23), 10 (Nov. 30), 20 (Dec. 29), and 126 (Jan. 4). In time and in numbers this series of collections can be divided conveniently into two collection groups, group 1 comprising the 150 specimens taken from October 1 through November 30, and group 2 comprising 146 specimens taken on December 29 and January 4.

The 296 specimens of collection groups 1 and 2 were measured to the nearest one-half millimeter, aged by the scale method, and sexed by dissection. In Table 9 is shown a partial summary of calculations broken down according to collection groups, age-groups and sexes. In Fig. 12 is presented a comparison of the mean standard lengths of the sexes between collection groups within the different age-groups using the method of Hubbs and Perlmutter (1942) that enables one to

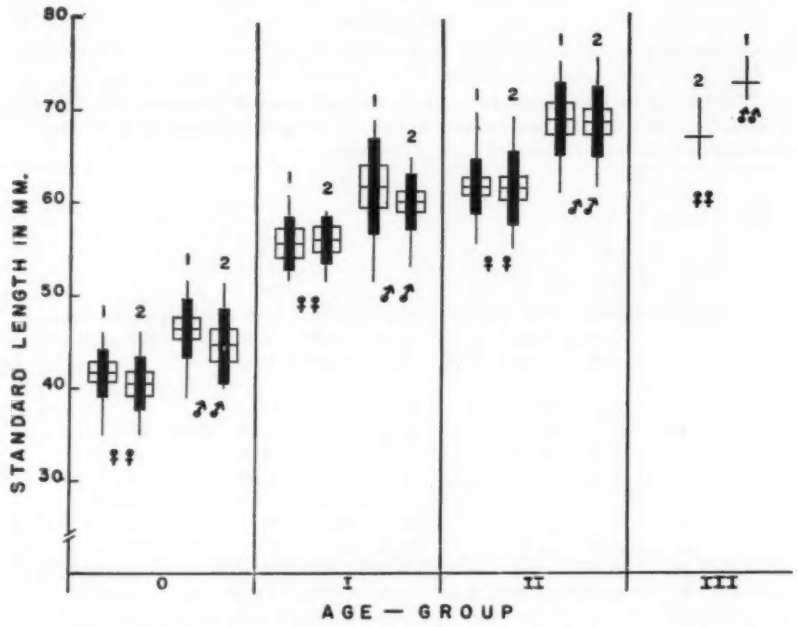

FIG. 12. Comparison of the mean standard lengths of the sexes between collection groups 1 and 2 within different age-groups in *Etheostoma b. blennioides*. Collection group 1 represents the specimens taken from October 1 through November 30, 1949, collection group 2 are those fish taken on December 29, 1949 and January 4, 1950. Explanation of sample comparison diagrams in text.

judge the significance of differences between means by inspection. In each of the vertical figures the range of variation is indicated by the vertical line; the mean is represented by the single horizontal line; one standard deviation on either side of the mean is marked by the solid rectangle; and twice the standard error on either side of the mean is indicated by the hollow rectangle. In a comparison of two sample means no significant difference between them is indicated if more than one-third of the length of the shorter of the two hollow rectangles is overlapped by the longer. A comparison between collection groups of the same sex within any age-group shows that one-half or more of the shorter rectangle is overlapped by the longer. Therefore, it is indicated that in any age-group there is no significant difference between the mean standard lengths of the same sex when collection group 1 and 2 are compared.

Further examination of the samples shown in Fig. 12 shows that there is no overlap of corresponding hollow rectangles between males and females of the same age-group, regardless of collection groups. When there is even a slight separation between the hollow rectangles of any two samples, a considerable reliance can be placed on the significance of the difference between the sample means. Fig. 12 clearly demonstrates that there is a highly significant difference

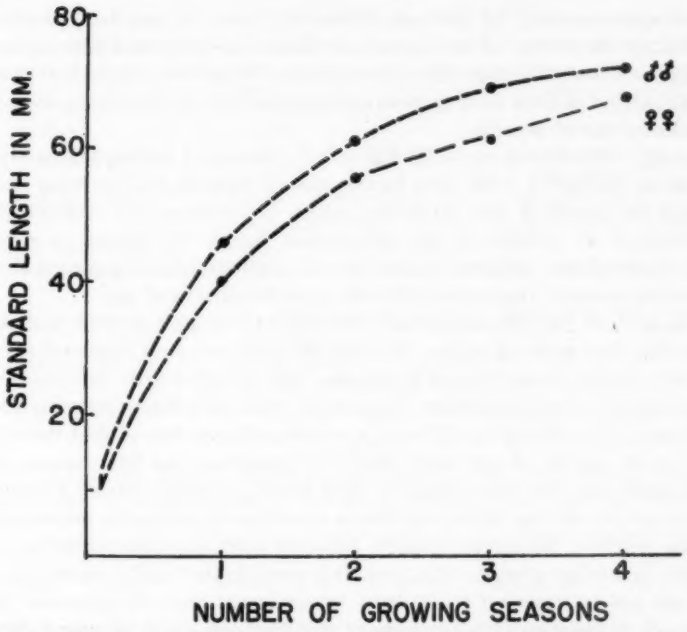

FIG. 13. Comparative growth rates of 149 male and 146 female *Etheostoma b. blennioides* through four growing seasons. Points of the graph are the mean standard lengths of the sexes for each age-group according to figures of Table 9.

between the mean standard lengths of males and females in age-groups O, I, and II. This significant difference is always in the same direction, that is, the males are larger than the females. (The paucity of specimens of age-group III in these collections prohibits a similar statistical treatment, but the means of the few specimens available indicate that the same condition persists in that advanced year.)

In Table 9 collection groups 1 and 2 have been combined. In Fig. 13 the curves show the comparative growth rates of the males and females for four growing seasons as represented in the total collection of 296 greenside darters. The vertical distances between the sexes at the different points represent the significant differences between the mean standard lengths. It is apparent from Fig. 13 that males grow faster than females and also attain a larger absolute size. Lachner *et al.* (1950) came to the same conclusion in their growth study.

If the percent of growth attained at the end of a growing season is derived as that ratio between the mean standard length of an age-group and the mean standard length of age-group III, it is indicated that males and females attain approximately the same percentage of growth in each year. Thus, males with a mean standard length of 45.7 mm. at the end of the first growing season had attained approximately 63 percent of total growth, and females at 41.2 mm. had

attained approximately 61 percent. Males 60.7 mm. in standard length had reached about 84 percent of total growth at the end of the second growing season, and females at the same stage had attained about 82 percent. Males had attained about 95 percent of their total growth at the end of the third growing season and the females about 91 percent.

Longevity.—The oldest male (72.0 mm. S.L.) observed during this study was collected on January 4, 1950 after having passed through four growing seasons. Assuming the month of June to be the month of hatching, this individual was approximately 43 months of age. The oldest female (71.5 mm. in standard length) observed was collected November 12, 1949 after having passed through five growing seasons. This individual was about 53 months of age.

In Table 9, of the 296 specimens collected 119 or 40.2 percent had passed through the third growing season, but only 10 specimens or 3.3 percent survived the fourth, and of these 10, one female had survived the fifth. Such an abrupt decline suggests a heavy mortality between the third and fourth growing seasons. Field observations during the different spawning seasons showed that these larger fish (34 to 36 months of age) were relatively numerous, but late summer collections yielded very few individuals in their fourth growing season. Presumably most darters of this age die in the weeks immediately following termination of spawning activity. No direct evidence has ever been available to verify a mass mortality in this age-group at this time. It is possible that such a mortality could occur and not be observed for at least two reasons: first, the greenside darter lacks an air bladder and the carcasses of the dead fish could become lodged beneath stones out of sight of an observer; second, such carcasses would be disposed of quickly by the very dense population of scavenging crayfish.

It is concluded that in the greenside darter population of Salmon Creek the typical life span for both sexes is three years (approximately 36 to 39 months). A few individuals of both sexes survive the fourth growing season. Only one female was found that survived a fifth. Lachner *et al.* (1950: 101) also found one female that survived the fifth growing season.

Relationship of comparative growth between sexes to fecundity and parental care of eggs in percid fishes.—Some species closely related to *blennioides* construct nests, or choose nesting sites, for their eggs, and the eggs are guarded by the male. Such percid fishes include *Etheostoma nigrum* (Seal, 1892: 9), *E. f. flabellare* (Lake, 1936: 819–20, 829), and *E. maculatum* (Raney and Lachner, 1939: 162), *E. punctulatum* (J. Petravicz, 1936: 80–82) lays its eggs on submerged vegetation or other objects in the water. *Percina caprodes* (Reighard, 1913: 104) and *Hadropterus maculatus* (W. Petravicz, 1938: 42–4) bury their eggs in the sand. These three species afford their eggs little or no protection. *E. b. blennioides* deposits its eggs on the green alga, *Cladophora*, more or less buried among the many filaments of the algal growth, and no parental care by either sex was observed during three breeding seasons.

Raney and Lachner (1939: 236–37), summarizing from the available literature, stated that in those species where a territory is guarded the males average larger than the females, and in those percid fishes not guarding nests the sexes are

TABLE 10

Summary of available data in the literature pertaining to the relationship of comparative growth between sexes and fecundity of those percid fishes affording parental care to eggs

Species	Growth of Male to Female	Number Eggs Per Female
Etheostoma f. flabellare	Larger. (Lake, 1936: 828).	422 (50.0 mm. S.L.). (Lake, 1936: 825).
Etheostoma maculatum	Larger. (Raney and Lachner, 1939: 164).	400 (50.0 mm. S.L.). (Raney and Lachner, 1939: 162).
Etheostoma caeruleum	Larger. (Reeves, 1907).	—
Etheostoma nigrum olmstedi	Larger. Faster. (Raney and Lachner, 1943: 237).	360. (Hall, 1935: 53).
Etheostoma n. nigrum	(Presumably same as *E. n. olmstedi*.)	395. (Hall, 1935: 53).
Etheostoma longimanum	Larger. Faster. (Raney and Lachner, 1943: 237–38).	—

TABLE 11

Summary of available data in the literature pertaining to the relationship of comparative growth between sexes and fecundity of those percid fishes affording no parental care to eggs

Species	Growth of Male compared with Female	Number Eggs Per Female
Stizostedion v. vitreum	Smaller. (Eddy and Surber, 1947: 212).	26000 per lb. of fish. (Eddy and Surber, 1947: 212–13).
Perca flavescens	Smaller. (Raney and Lachner, 1943: 237).	35000 (250 mm. in S.L.). (Hall, 1935: 51).
Hadropterus maculatus	Equal. (W. Petravicz, 1938: 40).	—
Percina caprodes	—	3172 (5 inch adult). (Evermann and Clark, 1920: 429).
Etheostoma microperca	—	200 plus. (J. Petravicz, 1936: 81).
Etheostoma b. blennioides	Larger. Faster. (Lachner *et al.*, 1950: 107–08.)	—

approximately equal in size or the females are larger. Hall (1935: 77) studied 36 species representing many families from the fecundity-nurture standpoint. He found that those species producing fewer eggs afforded more parental care than those producing large numbers of eggs. Tables 10 and 11 have been constructed to show comparative growth between sexes and fecundity of the species in the presence or absence of parental care for the eggs among percid fishes as determined from the available literature.

In Table 10 are shown the comparisons among the percid fishes (in this table all darters) affording parental care to eggs. In each case where data are available the males are larger than the females and the number of eggs per female per breeding season is low. Here the postulate of Raney and Lachner is supported, as are the findings of Hall. In Table 11 are shown the comparisons among percid fishes in which no parental care is afforded the eggs so far as is known. The postulate that in those percid fishes not guarding eggs the sexes are about equal in size, or

the female is larger, is supported by three species of Table 11, namely *Stizostedion v. vitreum*, *Perca flavescens*, and *Hadropterus maculatus*. The greenside darter affords no protection to its eggs, yet the males are larger than the females. Hall's conclusions that fishes laying large numbers of eggs tend to afford no parental care to them is supported by all species of Table 11 except *E. microperca*. The extremely small size of this darter may account for the low number of eggs deposited.

ACKNOWLEDGEMENTS

I am indebted to the late Professor Sherman C. Bishop for the suggestion of this study and for his guidance and encouragement during the course of the investigation. I express my sincere appreciation to: Professor Donald R. Charles, for valuable criticisms and co-operation during my study; Dr. S. Lee Crump, for direction in statistical treatment of data; Dr. Babette I. Brown, for the identification of the green alga, *Cladophora;* Professor Edward C. Raney, for the loan of specimens and advice and encouragement through the years; Dr. Frances N. Clark, for a description of measurement techniques on fecundity problems.

I am grateful to Carolyn E. Fallon, artist, for the drawings and illustration work. Mr. Raymond J. Maas produced the photomicrographs of the fish scales and co-operated in many technical capacities. In a large part this study has been completed because of the assistance of many people in collecting specimens, and the consent to the right of trespass by landowners along the course of Salmon Creek.

Mr. William A. Ellison, Jr. eased my research schedule so that the final draft of the manuscript could be made. Dr. Ernest A. Lachner critically reviewed the manuscript.

SUMMARY

A synonymy of *Etheostoma blennioides* is given, and specific characters are mentioned. Color descriptions and patterns are described in detail for adults and juveniles throughout the year. The general distribution for the species, and the northern subspecies, *E. b. blennioides* in particular, is presented. The topography of Salmon Creek, Monroe County, New York, is discussed. The greenside darter is found to be primarily a riffle dweller in all age-groups and in both sexes. During the colder months of the year juveniles and age-group I females seek quiet water to some extent. There appears to be a segregation of males within the riffle areas, the larger in deeper, swifter water near the largest rocks. Previously noted association of the species with green alga is confirmed: in Salmon Creek the alga is *Cladophora*. Certain fishes are found with the greenside darter more consistently than others in the riffle crest area. No predators are identified that actually prey upon the greenside, but the most vulnerable spot in the life history of the darter is apparently the egg stage where predation by an unknown agent was observed.

Locomotion is accomplished chiefly by the tail and sometimes by the pectoral fins alone. Jets of water expelled through the gill slits might be responsible for

forward movement at certain times. The resting position of the greenside darter is described.

Both sexes feed throughout the year, including the spawning season; less feeding occurs in the colder months than in the warmer. Insects form almost the entire diet.

The species is found to be very sensitive to white or bright red light, movements by an observer, movement of food, and jarring of the container.

Both sexes mature and spawn in the spring of the year following that of hatching. Mean standard length of males at this time is 45.7 mm. (range = 39.0 to 51.5 mm.), and that of females is 41.2 mm. (range = 35.0 to 46.0 mm.).

Sexual dimorphism in the species is most apparent in coloration and pattern. Sex ratio studies show a 1:1 ratio to exist in Salmon Creek.

The value of the study of the distribution of ova-diameters in fishes when the life history is unknown is demonstrated. The study indicates the number of eggs spawned per female of each age-group during a spawning season; subsequently these numbers were verified by pre-spawning and post-spawning season examinations of ovaries of females of different age-groups and by the actual count of eggs spawned by captive females in the laboratory. The ovaries as indicators of the length of spawning season in freshwater fishes are discussed.

Spawning is initiated in the Salmon Creek population of greenside darters when the water temperature reaches 51°F. and is maintained at that point for a few days. This occurs in mid-April and spawning continues to mid-June. According to observations spawning is restricted almost exclusively to hours of darkness. Water temperature influences spawning, and a drop in water temperature below the critical 51°F. can cause the cessation of spawning activity. The eggs of the greenside are laid upon the filaments of the green alga, *Cladophora*, and no other place in Salmon Creek. The spawning act as witnessed in the laboratory occurs once to perhaps five times a spawning night and approximately 40 eggs to an act are deposited (range = 2 to 192 eggs); the data are based upon age-group III fish for the most part. An estimate of approximately 37 acts per spawning season per female has been computed. The courtship behavior attending the spawning act was observed and one instance of possible courtship activity in the stream has been described. Individuals of the species are promiscuous. After the spawning season most activity in both sexes is concerned with feeding. Loss of most of the brilliant coloration occurs at this time, particularly in the females.

The eggs of the greenside darter are spherical, transparent, demersal and adhesive. A prominent yellow oil droplet is present. The average diameter is 1.85 mm. The embryo is figured and described from the 2-cell cleavage stage to the time of hatching. Hatching occurs 18 days after fertilization with water temperature at 55° to 58°F. Emergence from the egg capsule is tail-first. The prolarva requires 6 days to absorb the yolk sac, with water temperature at 60°F. The prolarva is figured and described. Postlarvae were successfully fed and brought through the 10 days following yolk sac absorption. The postlarva is described through this period and figured on the tenth day. Transition of postlarva to juvenile occurs in July, possibly late June.

Age determination was accomplished by the scale method. Cutting over marks in the lateral fields and delimitation of areas of differentially spaced circuli in the anterior field were established as true year marks or annuli. Males grow faster and attain a greater length than the females. At the end of the first four growing seasons males average as follows: 45.7, 60.7, 68.7, and 72.5 mm. in standard length, respectively. The females at the same time average 41.2, 55.8, 61.4, and 67.8 mm. in standard length. At the end of each year the differences between the mean standard lengths of the sexes are statistically significant. Both sexes attain over 60 percent of total growth in the first growing season. Typical life span of the species is three years (36 to 39 months), but a few individuals may survive the fourth or fifth growing season.

A survey of the literature was made to study the relationship of the comparative growth between sexes to fecundity and parental care in the percid fishes. *Etheostoma b. blennioides* was placed with those species producing relatively large numbers of eggs and affording no parental care to the eggs, although the males grow larger and faster than the females. This latter characteristic is more frequently associated with percid fishes producing relatively few eggs and affording parental care to them.

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STRUCTURE AND RELATIONSHIPS OF THE ANGLERFISH, LOPHIUS AMERICANUS

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The specimen dissected and illustrated in this study is an adult female Angler weighing 25 pounds, total length 660 mm., taken in late March, 1954, at Core Point in Pamlico Sound, Beaufort County, North Carolina. It was brought to East Carolina College through the courtesy of Dr. Christine Wilton. The investigation does not include the entire anatomy of the fish, but is limited primarily to the skeletal and muscular specializations concerned with locomotion, and the use of the remarkable "fishing rod" on the head. Miscellaneous other observations are added, which have general interest or shed light on the relationships of *Lophius* to other fishes.

Gregory (1933) illustrated the skulls of *Lophius* and a number of other Pedicu-

late fishes. He discussed the position of *Lophius*, inferring that it is fairly primitive among Pediculati but apparently derived from some Antennariid, while the order as a whole appears to be closely related to the toadfishes (Batrachoidea). Again in 1936 (Gregory and Conrad) and 1951 (Gregory) he discussed the problem, with reference to the adaptive branching of the Pediculati. With most of his conclusions, so far as the present study gives evidence, we agree, but would suggest that *Lophius* is too primitive in certain respects to be derived from any Antennariid, as shown in the section on relationships. That the Angler and other Pediculate fishes actually use the lure in fishing is demonstrated in recent papers by Gudger (1945a, 1945b, 1948). Other sources employed in this study are listed in the bibliography.

I. The Pectoral "Limb" and its Muscles

The name Pediculati, for the order to which the Angler belongs, refers to the leg-and-footlike form of the pectoral fins, and, to a lesser extent, of the pelvics, and the accompanying mobility of these fins. The fish *walks* with its paired fins rather than using them as blades or fans in ordinary swimming; this is especially true of *Lophius*, the sea-mice (Antennariidae), and the sea-bats (Ogcocephalidae). In the pectoral fin, but not the pelvic, the radial bones are greatly lengthened, so that the fin proper is supported on a mobile leg, and the muscles are elaborately specialized.

Lophius, while at rest on the bottom, holds its pectorals with the "leg" projecting back and slightly outward, and the fin in a horizontal plane with the rays extending laterally. This position does not correspond to that of an ordinary teleost pectoral fin held horizontally, for a normal teleost has the strong, stiff cutwater edge of the fin turned forward and up, whereas in the Angler this primitively *anterior* edge is behind, and, in fact, turned down. Thus the apparent dorsal surface of the fin (dark-colored like the upper parts of the body) is the primitive ventral surface, while the original dorsal surface has been brought

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Fig. 1. Right pectoral fin and radials from below, and radials from above. Fig. 2. Left pectoral fin muscles from below. Fig. 3. Left pectoral fin muscles from above. AB—abductor basalis. ABI—adductor basalis inferior. ABM—adductor basalis medialis. ABS—adductor basalis superior. DBS—depressor basalis superficialis. ER—extensor radialis. FRI A, FRI B—flexor radialis inferior, A, B. FRS—flexor radialis superior. Fig. 4. Pelvic girdle and fin muscles, and pectoral girdle, from below. ADS—abductor dorsalis superficialis. AP—adductor posterior. AVP—abductor ventralis profundus. AVS—abductor ventralis superficialis. CL—cleithrum. CO—coracoid. DL—depressor lateralis (pelvis). DM—depressor medialis (pelvis). DP—depressor profundus (fin). DS—depressor superficialis (fin). HIA—hyohyoideus inferior anterior. HIP—hyohyoideus inferior posterior. L—levator (fin). PL—longitudinal beam of pelvis. SC—scapula. Fig. 5. Fourth dorsal spine and pterygiophore, with positions of muscles indicated; arrows show direction of muscle action; right side. D—outline of depressor. E—outline of erector. I—insertion of inclinator. Fig. 6. First two dorsal spines and pterygiophore, with positions of muscles indicated; arrows show direction of muscle action; right side. D1—depressor of first ray. D2—depressor of second ray. E1—erector of first ray. E2—erector of second ray. I2—insertion of inclinator on second ray. P1—first part of pterygiophore. P2—second part of pterygiophore. R—retractor.

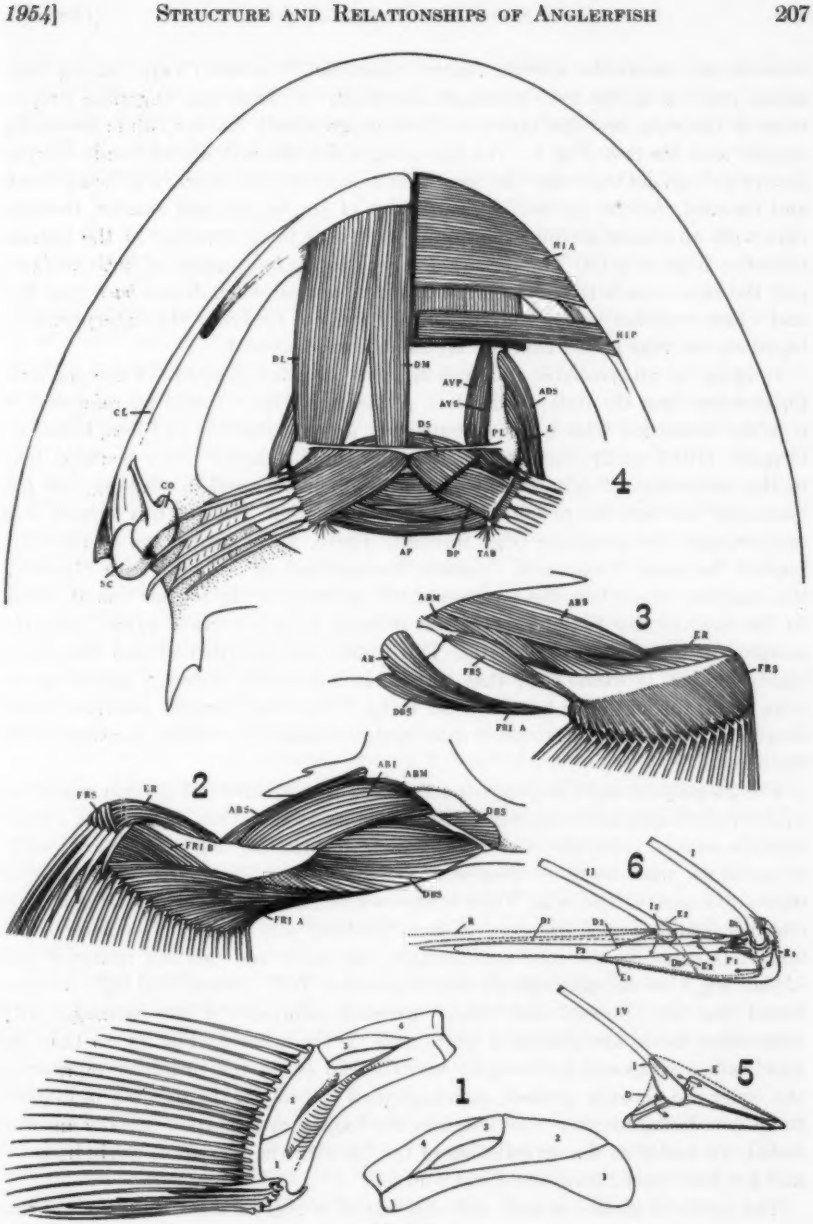
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FIG. 1–6.

beneath and shows the whitish ventral coloration. The last 7 rays (taking their actual position in the fish) terminate externally in roughened fingerlike projections of the skin, and the last 4 or 5 are progressively shorter, while becoming stouter than the rest (Fig. 1). The line along which the rays are set bends sharply downward, giving this edge the form of a scoop with the concavity facing down and forward. At the (actual) forward edge of the fin we find slender, delicate rays with no special modification, quite like those to be expected at the normal posterior edge of a fin. The direction in which the fin-muscles of both surfaces pull the rays is such that the rays extend and spread when drawn *back*, but flex and close together when moved *forward*, which confirms the interpretation based on the rays alone, that the fin has become inverted.

Judging by all available illustrations, this inverted position of the pectoral fin characterizes the order Pediculati, although we have found no reference to it in the literature. This is not a completely unique situation in fishes, however; Gregory (1951, p. 211) recognized that the three separate lower pectoral rays in the sea-robins (*Trigla*, *Prionotus*, etc.), which are used in walking, are not "preaxial" but are the posterior rays. The fin in that case too has turned over and brought the posterior edge forward. There, is, of course, no relationship implied between *Trigla* and *Lophius*. Comparison of *Lophius* with *Opsanus*, the toadfish, shows how the position of the pectoral in the former was attained. In the toadfish the broad base of the pectoral is in a vertical plane, with the morphologically anterior edge up. The radials are lengthened and the fin is highly mobile. It seems clear that to turn its lower edge forward and its upper edge back, through about 90°, would bring it into the *Lophius* position. Other details will be given later to show that *Opsanus* stands near to the ancestry of the Pediculati.

The 26 pectoral rays in *Lophius* are forked basally and rest astride a piece of rubbery (collagenous) connective tissue, not cartilage. This in turn fits loosely over the convex outer rim of the radials, which form the "leg". These are largely of cartilage, with no more than a superficial layer of bone around the middle parts, and none at the ends. They comprise two main pieces, which Gregory and Conrad (1936) termed, for convenience, "radius" and "ulna". The latter is the larger. It supports the rays and occupies the inner and (actual) posterior side of the "leg," but morphologically this is anterior. With transmitted light we have found that the "radius" and "ulna" are each composed of two cartilages with connective tissue showing as a white zone in the sutures (Fig. 1), so that the four radials supposed by Gregory and Conrad to be the primitive number in the order are actually present, although they considered the number in *Lophius* to be two. Using number 1 to designate the longest and morphologically anterior radial, we find that the articulation of the fin with the scapula is made by no. 1 and 4, while 1 and 2 form the distal border.

The pectoral girdle on each side consists of a big, scimitar-shaped cleithrum (Fig. 4) with a movable symphysis anteriorly and an abrupt angle posteriorly, armed with a forked spine (probably fused postcleithrum), a loose supracleithrum (not shown) articulating with the skull, and a greatly reduced scapula and

coracoid. Only the scapula articulates with the radials; it is globose and entirely of cartilage. The coracoid is superficially bony and has a narrow bony bridge extending antero-laterally to meet the edge of the cleithrum. A small foramen passes between scapula and coracoid.

The normal, primitive pattern of pectoral fin muscles in a teleost is this:

Dorso-medial Levators	Superficial, to rays
	Deep, to radials
Ventro-lateral Depressors	Superficial, to rays
	Deep, to radials

These originate on the pectoral girdle and show little subdivision, except where their insertions occur on the separate rays. It is extremely difficult to apply this pattern to *Lophius* for two reasons: (a) the inversion of the fin, and (b) the occurrence of 11 separate muscles on account of increased mobility and free jointing of the fin skeleton. Further, it would lead to useless confusion if the term *depressor* were applied to muscles which have, by turning over with the fin, become *levators*, and vice versa. It seems better to classify them strictly according to their existing locations and functions, and then indicate their derivation. This can be done without question for all which insert on the fin-rays, but the original arrangement of those which run from the pectoral girdle to "radius" or "ulna" must be taken as provisional until their larval condition can be studied. The adjective *basalis* will be used for each muscle inserting wholly on "radius" or "ulna," and *radialis* for each one inserting wholly or primarily on the fin-rays.

1. *Depressor basalis superficialis* (Fig. 2). Seen from below, this is a large spindle-shaped muscle on the outer side of the basal joint. At its origin it is partly split, attaching first to the outer angle of the cleithrum and ventral half of the coracoid arch, and second, passing through the arch and attaching to the cleithrum. The insertion is in the shape of a long triangle on the proximal half of the "ulna." The muscle depresses and slightly abducts the fin as a whole.

2. *Depressor basalis profundus*. This is not visible in the figure, being covered externally by the previous muscle. It is smaller and deeper than *D. b. superficialis* but runs nearly parallel to it. The origin is on the ventral side of the scapula, the insertion the ventral surface of the shaft of the "radius." Action: depresses the fin.

3. *Adductor basalis inferior* (Fig. 2). As seen from beneath, this is on the medial side of the basal joint, and nearly as large as *D. b. superficialis*. Its origin is almost a point on the ventral face of the coracoid and beginning of the coracoid arch. The insertion is in a triangle on the medial face of the "ulna." Action: adducts and slightly depresses the fin as a whole.

4. *Adductor basalis medialis* (Fig. 2, 3). This is a smaller, deeper muscle than the preceding, lying next to it dorsally. It originates on the medio-ventral face of the coracoid and inserts on the shaft of the "radius" on the side facing the "ulna." Action: adducts the fin and apparently rotates the lower surface outward.

5. *Adductor basalis superior* (Fig. 2, 3). This is the most medial and dorsal of the "basal" group of muscles. Its origin is on the ventral surface of the postclei-

thral spine at its base, and the insertion is by a short tendon on the medial edge of the "ulna," directly distad from *Add. bas. inferior*. The action is straight, horizontal adduction of the fin.

6. *Abductor basalis* (Fig. 3). This is a short muscle originating on the dorsal side of the angle of the cleithrum, and inserting along the outer edge of the "radius." Action: to abduct the fin as a whole.

7. *Flexor radialis superior* (Fig. 2, 3). This is a superficial muscle on the upper surface, which inserts by separate short tendons on all 26 rays. The direction of pull is such as to flex rather than elevate the rays, although a strong pull will do the latter. (Twisting, raising, or lowering the fin is accomplished in *Lophius* by the preceding "basal" muscles rather than by those inserting on the rays.) Flexion in this case is a forward movement of the rays, by which the angle between them and the "leg" is decreased, and the rays are also brought closer together, as in closing a fan. This muscle originates by a long tendon to the dorsal side of the scapula; the more distal fibers of the muscle attach to a fascia, as shown, which is the farther end of the tendon, while proximally the fibers form a spindle-shaped bundle along the tendon.

8. *Extensor radialis* (Fig. 2, 3). This is partly covered by, and is thicker than, the previous muscle. Its origin is on the distal two-thirds of the upper surface of the "ulna." It inserts on a series of small pointed flanges near the bases of all the fin-rays; these flanges project backward (caudad), each one somewhat overlapping the base of the next ray behind (see the lower rays in Fig. 1). The effect of pulling on the flanges in the direction of the muscle-fibers is to extend or spread the rays laterally; a strong pull elevates them.

9. *Flexor radialis inferior A* (Fig. 2). Although this muscle inserts primarily on the 20 most anterior fin-rays, on the lower side, by short tendons, it has also a fleshy insertion on the lateral edge of the "radius," probably secondarily. Its origin is by a strap-like tendon on the lateral face of the scapula. It is a broad, triangular, superficial muscle on the downward side of the fin, and the action is mainly to pull the rays forward and together (flexion), with a slight tendency to depress the fin.

10. *Flexor radialis inferior B* (Fig. 2). Partly covered by the preceding, this is obviously a smaller division of the same flexor muscle, inserting on the last 6 rays of the fin (morphologically the first 6). Since these rays belong to the part of the fin which curves under, the direction of the muscle fibers is somewhat different, but the effect is to pull the rays forward in a cupping action with the others. The muscle originates on the bony ridge of the "ulna," seen in Fig. 1.

11. *Depressor radialis*. This muscle is entirely concealed by the two preceding and is not shown in the figure. It originates in the groove of the bony ridge on the lower side of the "ulna" (Fig. 1), and inserts (a) along the whole length of the connective tissue saddle which supports all the rays, and (b), more strongly, on the lower tips of the rays themselves. Its depressor action is probably synergistic with the flexion produced by the two previous ones, together resulting in a strong thrust forward and down, which would throw the fish back and upward in the water.

In regard to the derivation of these muscles from something like those found in a "normal" teleost, we can be quite certain that the *flexor radialis superioris* and *extensor radialis* belonged, originally, in the ventro-lateral depressor group and were superficial, going to the rays. The two divisions of the *flexor radialis inferioris*, and the *depressor radialis*, are equally certainly derived from the primitive superficial part of the dorso-medial levator group. The reason is that the insertions on the rays would not be changed by the inversion of the fin, and therefore the muscles (at their insertions) would accompany the sides to which they originally belonged. The six "basal" muscles were originally either deep levators or deep depressors, but we cannot be perfectly sure of their distribution. It seems likely, from their insertions and present action, that the *depressor basalis superficialis*, *d. b. profundus*, and *abductor basalis* belonged with the ventro-lateral (depressor) group, and that the three adductors were dorso-medial levators.

II. THE PELVIC FIN AND ITS MUSCLES

The pelvic fins are between and anterior to the pectorals. In our specimen a line drawn between the posterior edges of the bases of the pelvics lies 75 mm. anterior to a similar line connecting the anterior edges of the pectorals. The fin has 6 rays, but as the first and second are closely joined there appear, externally, to be 5. These end in finger-like projections of the skin at the margin, and the fin appears fleshy. Thin ligaments between rays 2, 3, 4, 5 and 6 allow a spread of about 10 mm. in each interspace, but pull the rays forward as a group when the fin is abducted. Movements in any direction are extensive: adduction, abduction, elevation, depression.

Part of the mobility of the fins can be attributed to the pelvis and its muscles. The two pelvic bones meet each other at a median joint, and both articulate loosely with the cleithra anteriorly. Thus the pelvis makes a broad H, hinged against the pectoral girdle and lying within its wide arch. Therefore the pelvis and pelvic fins can be lowered, and the adjacent abdominal wall with them, in a manner analogous to dropping the landing gear of a plane.

Longitudinal muscles of the abdominal wall contain no myosepta and are reduced to a thin sheet in the ponderous abdominal region behind the fins. Medially, in fact, there is no muscle directly behind the pelvis, but more laterally some of the abdominal fibers converge to attach by a short tendon on the posterior angle of the pelvis (Fig. 4). Still more laterally the abdominal muscles, gaining in thickness, come forward past the sides of the pelvis and insert along the inner curvature of the cleithra anterior to the pectorals. Thus there is scarcely any direct elevating action on the pelvis as a whole; this function is probably rendered superfluous by the fact that the fish rests most of the time on the bottom, its weight counteracting the depressor action of the "landing gear" muscles next described.

As seen in Fig. 4, two pairs of flat but thick muscles run forward from (a) near the midline of the pelvis, and (b) the angle of the L formed by each pelvic bone; the origins are continuous with each other on the posterior face of each

cleithrum near the symphysis. These may be named *depressor medialis* and *depressor lateralis*, respectively, of the pelvis (not pelvic fin). They are, of course, longitudinal strips of the ventral abdominal musculature, analogous to the *rectus abdominis* of tetrapods, because they are morphologically anterior to the pelvic girdle. Anteriorly they are covered (ventrally) by the two transverse parts of the *hyohyoideus inferior*.

As in most fishes, there are fundamentally two groups of muscles inserting on the fin, *levators* dorsally and *depressors* ventrally. But in *Lophius* these are highly differentiated because of the added movements of adduction and abduction. Here we must include further details of the skeletal structure. The pelvis is partly bone but becomes wholly cartilaginous in the region of the fin-joint, where it forms a convex crescent-shaped knob. The long axis of this knob tilts slightly down and forward, in the same plane as the transverse axis of the fin. The six fin-rays insert upon a collagenous, fibrous piece of connective tissue which in turn fits like a cap on the convex knob of the pelvis, and slides freely there, in any direction. This *basal cap* seems to lack any true cartilage, and takes the place of the radials normally serving as supports for the fin. The rays fork widely at their bases, each branch attaching dorsally or ventrally on the basal cap. In the resting position they are directed back and out, as in Fig. 4. They are free to swing anteriorly (abduction) or posteriorly (adduction) at their articulation with the basal cap, and the latter, as already noted, can take part in this movement. Elevation and depression require movement of the cap, even though some of the muscles responsible for them may insert on the bases of the rays. On the dorsal side at the base each ray (including the last) carries a pointed flange directed back, overlapping the next behind (except, of course, that the last one does not overlap anything). These flanges in part control the positions of the rays in series by keeping them interlocked, and in part provide leverage for the dorsal adductor muscle which inserts on them.

The *ventral, depressor* group of fin muscles is as follows:

1. *Depressor superficialis* (Fig. 4). An external sheet inserting on a fascia connecting the bases of the fin-rays; the origin is on the ventral side of the median portion of the transverse bar of the pelvis, except that a small part comes through a gap between the bone and a transverse ligament, and continues across the midline on the anterior side of the bar, uniting with the opposite *d. superficialis*.

2. *Depressor profundus* (Fig. 4). This is thicker than the above, and inserts on the basal cap which supports the fin-rays. The origin is in the hollowed posterior surface of the transverse pelvic bar.

3. *Abductor ventralis superficialis* (Fig. 4). This and the following, derived from the depressor group, appear as one muscle at their origin on the median face of the longitudinal beam of the pelvis. They come posteriorly through a gap between the pelvis and a transverse ligament, and here split, the *A. v. superficialis* inserting at the base of the first ray.

4. *Abductor ventralis profundus* (Fig. 4). This slip, originating with the previous muscle, inserts on the anterior ventral part of the basal cap. Acting as synergists with the dorsal abductor, these muscles move the fin laterad and forward, and spread its rays.

The *dorsal, levator* group of fin muscles is the following:

1. *Levator* (Fig. 4). This is a thick sheet, originating on the dorsal side of the longitudinal beam of the pelvis, and inserting on the dorsal margin of the basal cap.

2. *Abductor dorsalis superficialis* (Fig. 4). This originates, as shown, on the ventrolateral face of the longitudinal beam, about halfway forward, and inserts on the anterior face of the first ray. It is evidently derived from a superficial part of a primitive levator mass. By pulling forward on the first ray it abducts and spreads the whole fin.

3. *Abductor dorsalis profundus* (not shown). This originates along the dorsolateral surface of the longitudinal beam, and inserts on the bases of the first three rays. It is probably a synergist of the preceding, indirectly pulling forward the basal cap.

4. *Adductor anterior* (not shown). This is a deep, dorsal muscle originating on the dorsal side of the transverse bar of the pelvis, near the midline. It inserts on the pointed flanges of the rays, previously described. The action is primarily to adduct the rays directly, but this does not seem to be very effective without the complementary action of the next one.

5. *Adductor posterior* (Fig. 4) This peculiar group of transverse bundles crosses the midline in a horizontal plane, and attaches in part to the flanges of the fin-rays. As a group they are barely separate from the previous muscle, but their action is much more clearly adduction. The slips from opposite sides interdigitate as they cross over, especially in the posterior part of the group. Those that insert on the fin rays on one side have their origins on the posterior face of the pelvis on the opposite side.

III. MECHANISM OF THE DORSAL SPINES

Six spiny dorsal rays are present in *Lophius americanus*, three on the head and three on the trunk, the first one being the famous "fishing rod." No membrane connects any of the six in our specimen, although illustrations sometimes show a low fin supported by the trunk spines. The skeletal support and muscular mechanism of the three trunk spines is essentially like that in most teleosts (Eaton, 1945). We figure the first of these, or number 4 in the whole series (Fig. 5). The pterygiophore is a narrow triangular piece, largely of cartilage, resting on its hypotenuse in the groove between the two lateral masses of trunk muscles. Each side of the pterygiophore bears a low bony keel slanting forward, above which is the area for the origin of the *erector* muscle, while below and behind is that for the *depressor* of the ray. The ray itself is swollen and forked basally, standing astride a low saddle on the posterior edge of the pterygiophore. The strap-like inclinator muscle inserts just above the base, at the spot indicated, and runs laterad to its origin on the fascia of the longitudinal muscles. Using this ray as a basis for comparison, we can interpret the structure of those on the head.

The third ray (not figured) rests firmly on the supraoccipital, with a reduced and immovable pterygiophore attached there by connective tissue. Its muscles are essentially as described above.

The first and second rays, 13 and 15 cm. long respectively, arise close together from one skeletal piece (Fig. 6) near the front of the head, and the first carries a leaflike flap of skin, the illicium, 2 cm. in length, which acts as a lure to attract prey (Gudger, 1945a, b, 1948; Bigelow and Schroeder, 1953). As would be expected, the pterygiophore has been formed by the union of two (those for the first and second rays), and the suture can be distinguished (*s* in Fig. 6). Ptergiophore 1, then, bears a median keel ventrally, and runs back under 2 as shown; pterygiophore 2 is flat, splint-like, and mostly cartilaginous. The block so formed, 70 mm. long, is not attached to the skull but fits loosely in a shallow groove over the middle part of the frontals and ethmoid. It can slide a few millimeters forward and back.

The first ray, which bears the illicium, stands on an upright keel at the tip of the pterygiophore. This keel has a transverse foramen through it and is therefore ring-shaped. The ray, forked basally, has a cross-ligament between its two feet, passing through the ring and thus anchoring it strongly while allowing great freedom of movement. There are no *inclinator* muscles for the first ray, but each *erector*, inserting on an anterior knob at either side above the base of the ray, passes down, around a connective tissue pad which acts as a pulley, and back along the lower edge of the keel where its fibers take origin, for 33 mm. The *depressors* are even larger; they run straight back, past the second ray, and originate on the posterior half of the pterygiophore block.

The second ray and its muscles are less highly specialized. It stands on a low keel but without a ring-lock. Its *erectors* and *depressors* originate on the sides of the pterygiophore in triangular areas as illustrated. In lateral view they are concealed by the *erectors* and *depressors* of the first ray. There is a normal *inclinator* on each side, with its insertion on the second ray as shown, and its origin on the frontal bone. But in addition two pairs of special muscles are provided which seem, from their location and attachments, to have been derived from the *inclinators*. (This might be verified by dissection of a larval specimen, not now available to us.) A long strap-like muscle, inserting by a short tendon close to the *inclinator* on the second ray, runs back 100 mm. and takes origin *behind the third ray* on the supraoccipital; this (pair) evidently *retracts* the pterygiophore as a whole. There is also a pair of protractors, originating on the frontals just under the origins of the *inclinators*, and running back obliquely to insert on the lower side of the flattened part of the pterygiophore. Thus almost any conceivable motion of either the first or the second ray is provided, either by their intrinsic muscles or by those moving the pterygiophore.

Here it is worthwhile to illustrate the astonishing lengths to which an adaptive mechanism, already highly specialized, may be carried, by referring to the rod and lure in a deep-sea angler, *Ceratias hölbolli*, as described by Bigelow and Schroeder (1953, 543–4). The male, being parasitic on the female, has no such device, but the female carries a single long, curved "tentacle" emerging from a pore on its head, capable of being thrust far up and forward, or withdrawn under the skin until the illicium hangs close over the mouth. Internally the "tentacle" attaches to a slender rod, the pterygiophore, which slides back or forward by means of retractor and protractor muscles. When retracted as far as possible, the

posterior end protrudes out of the back of the fish as a long fingerlike process; this in turn withdraws when the tentacle is protracted. Evidently fishing in the deep sea has been developed into a fine art by the Ceratioids.

IV. NOTES ON THE VISCERA

The *ovary* of our specimen was a single immense ribbon-like organ, intricately folded and supported by an extensive mesovarium. At the middle line, just above the rectum and at the posterior end of the body cavity, the peritoneum enclosing the ovary formed a short broad funnel opening to the exterior immediately behind the anus. When laid out straight, the ovary measured 517 cm. in length, and varied in width from about 8 cm., near the ends, to about 24 cm. medially. The *eggs* (estimated by counting weighed samples) numbered roughly 543,000, and occupied, in general, a single layer of translucent jelly, somewhat thickened along the edge.

Dorsal to the oviduct was a *urinary bladder*, pear-shaped, about 4 cm. in long diameter; laterally it received the mesonephric ducts, and opened externally by a minute pore at the posterior edge of the exit of the oviduct.

As Aristotle noticed, *Lophius* has its *gall-bladder* far removed from the liver, with a long cystic duct, and our specimen showed a second gall-bladder, almost as large, at the junction of the cystic and common bile ducts. There are two *pyloric ceca;* the *air bladder* is absent.

The *heart* is very large, consisting of four chambers in nearly linear series, dorsal to the depressor muscles which connect the pelvis with the cleithra. The sinus venosus receives, as usual, two hepatic veins posteriorly, the ducts of Cuvier postero-laterally, and a pair of internal jugular veins anteriorly. Between the internal jugular and the duct of Cuvier on each side there enters a subclavian. The auricle is normal, but the ventricle has a remarkably thin wall, and the bulbus arteriosus, in front of it, appears to have taken over the ventricular function of propelling the blood, as its lumen is nearly as large as that of the ventricle and its walls a great deal more muscular. The ventral aorta has become shortened so far that it gives rise only to the first pair of afferent arteries, while the second and third, on each side, come from a common stem opening separately from the side of the bulbus. There are three pairs of gills.

V. RELATIONSHIPS

The Pediculati have armlike pectoral fins, in which the anterior edge is brought around to the posterior side. The skin is soft and scaleless, the fins in many cases appearing fleshy rather than membranous. The more advanced Pediculates have lost the pelvic fins, but in those which still have them (Lophiids, Antennariids, Ogcocephalids) their appearance is even more handlike than that of the pectorals, although they lack the elongate radials. The gill-chambers are large, the six branchiostegals (5 in three advanced families) greatly lengthened, and the opercular flap carried back so far that the opening lies behind the pectoral fins. Most but not all Pediculates carry one or more dorsal fin-rays attached to the head, and the most anterior one is specialized to act as a lure.

Regan and Trewavas (1932) recognized three suborders of Pediculati: Lophioi-

dea, with *Lophius*, *Chaunax*, and the sea-bats (*Ogcocephalus*); Antennarioidea, the sea-mice (*Antennarius*, *Pterophryne*, and their relatives); and Ceratioidea, with a number of deep-sea families lacking pelvic fins. Among the latter, the males are sometimes reduced to miniature parasites on the females.

The nearest order to Pediculati appears to be that of toadfishes (Batrachoidea). Most of these also have soft, scaleless skin. The gill chambers are enlarged, and the radials of the pectoral fins lengthened, giving an internal similarity to the pediculate type of fin. The morphologically anterior edge of the pectoral is turned up, so that the plane of the base of the fin is vertical. The rays, in *Opsanus*, the common toadfish, are hidden in the skin so that the fin appears fleshy. *Opsanus* and *Lophius* show a considerable similarity in their skulls (cf. Gregory, 1933). Suborbital and nasal bones are absent, and the lacrimal is specialized. In *Opsanus* it has become long and splinter-like, closely appressed to the maxillary, while in *Lophius* (where the eye is much farther back from the mouth) the lacrimal is fused to the anterior end of the palatine, just overlapping the maxillary. The opercular and subopercular bones in these two fish are strikingly similar, those of *Lophius* being somewhat reduced. In *Opsanus* the gall-bladder is like that of *Lophius*, far from the liver, with a long cystic duct; at the junction of the cystic and bile ducts is a small glandular (?) pouch.

In contrast to *Lophius*, the toadfish shows the following characters which are common to most teleosts and therefore in this instance primitive. There is a lateral line with pores and head-canals, especially around the eyes. The nostrils are normal, and the large branchial opening is in the usual position, anterior to the pectorals. There are, of course, no fin-spines on the head. The first dorsal fin is separate and spiny (but reduced). Gill-rakers are present, although short. The air bladder is present, and abdominal muscles show myosepta.

In a few features, however, *Opsanus* must be regarded as specialized in comparison with *Lophius* and therefore not exactly on the line of structural ancestry of the Pediculates. It has no pyloric ceca, and the intestine is short and swollen. According to Regan (1912) the Batrachoids have lost the mesethmoid, and the epiotics are either lost or fused with the parietals. Undoubtedly *Opsanus* shows a specialization in its habit of laying a few large eggs attached to objects in sheltered places, while the numerous small eggs of *Lophius* are floated in a veil of mucus.

From such comparisons as these it may be correct to infer that the common ancestor of the two orders was a bottom-dwelling, soft-skinned, large-headed and large-mouthed fish, more like a toadfish than like the Pediculati, and probably coming within a broad definition of the Batrachoidea. *Lophius*, however, is said to occur as early as the Upper Eocene, so that there may have been approximately 50 million years for the adaptive radiation of the Pediculati.

Gregory and Conrad (1936) discussed the position of *Lophius* among the Pediculati, concluding that the most primitive condition of the pectoral fin in this order was probably that in Antennariidae and others which have three radials, and that the condition of two radials (*Lophius*, *Ogcocephalus*) arose by fusion of the middle with the lower piece. But in certain deep-sea Ceratioid

families (Ceratiidae, Melanocetidae, Gigantactinidae) four separate radials are present; Gregory and Conrad supposed that this happened by a secondary splitting of the lower piece in the 3-radiate forms. It is true that the three families named are extraordinarily specialized and should not be expected to show a truly primitive pectoral fin structure. But it is clear from our dissection that both "radials" of *Lophius* are compound, for sutures show that each of them arose by a union of two. This makes it quite evident that the primitive number in the order must have been four; we may add, however, that these four may not all have been separate and freely movable. Considering that the skeletons of the Ceratioids are characterized by delicate sliver-like bones which fail to develop normal breadth and the usual complex sutural contacts (for whatever adaptive reason), it may be that we see here an instance of delayed development applying also to the fins, so that the centers for the four radials appear, but they fail to progress to a stage of fusion. Phylogenetically, in terms of the adult only, this might perhaps be called a secondary splitting, but in developmental terms it constitutes a retreat rather than reversal. This is a possible explanation of the appearance of a "primitive" feature in an advanced line.

As to the dorsal spines, *Lophius* has six, and must in this feature be more primitive than other pediculates, which have 3, 2, 1, or none. Gregory (1933, p. 387–8) discussed the peculiar *Brachionichthys*, in which there are three head-spines connected with each other and with the soft dorsal by a fin membrane. There are no pelvics and the pectoral radials are two. The first head-spine is not modified as a lure. Gregory infers that *Brachionichthys* is probably the most primitive member of the order. In view of the evident relationship of Pediculati with Batrachoids, in which the first dorsal (usually vestigial) is not connected with the second, and the presence of six spiny rays in *Lophius*, which could scarcely be regarded as an increase over a primitive number, it seems that *Brachionichthys* must be specialized rather than primitive in having the fins united. In fact another Brachionichthyid, *Symplerichthys*, shows apparently an earlier stage in this secondary union, and also suggests that the first spine may have been simplified from an Antennariid-like illicium.

The habits and ecological situation of *Lophius* must be considered briefly in connection with relationships. As an adult it is adapted in many details of form and color for camouflage among seaweeds and rocks on the bottom. Although quite capable of active locomotion, its primary equipment is for waiting motionless. The great broadening and flattening of the head are a part of this. But during early development it is at first a minute, slender, pelagic larva, and then it enters a second larval stage (Eaton, 1942; Bigelow and Schroeder, 1953), quite different either from the first or from the adult, when it has expanded fan-shaped pectorals and pelvics, and lives concealed among floating seaweed. Here, possibly, is the real link with the sea-mice (Antennariidae) of similar habit. Although the latter include reef-dwellers as well as sargassum fishes, they creep around more actively than *Lophius*, and their camouflage is more specifically related to weed-hiding than to bottom-sitting. It seems likely, then, that *Lophius* has retained and exaggerated certain features of the sluggish Batrachoids, becoming more seden-

tary in its adult life, while in early development it takes on a temporary association with drifting weed. In some of its relatives an emphasis on this may have led to departure from bottom-dwelling altogether, with accompanying failure to complete the development of ancestral adult characters, and finally to abyssal life, while in another direction (batfishes) the flat bottom-dwellers practically eliminated the normal swimming equipment from their structure and became pedestrians.

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AN EXPERIMENTAL STUDY OF THE "BARRED" PIGMENT PATTERN IN MEXICAN AXOLOTL LARVAE[1]

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The experiments presented in the following were designed to expose the causal factors responsible for the arrangement of pigment cells in larvae of the Mexican axolotl, *Amblystoma mexicanum*. In this species of salamander the early larval pigment pattern has as its most striking feature the presence of from three to seven dorsoventrally oriented black bars consisting of aggregations of melano-

[1] This paper was presented on January 12, 1954 before the Elisha Mitchell Scientific Society at the University of North Carolina, Chapel Hill.

phores. Between these are alternating yellow bars of xanthophores. Initially three sources were considered as potentially responsible for this pigment cell pattern which hereafter will be referred to as "barring."

First, it seemed possible that the melanoblasts and xanthoblasts might have a metameric arrangement prior to their migration from the neural crest from which they arise. This possibility, which could account for barring in the most simple and direct manner, was discarded following observations on the initial appearance of melanophores and xanthophores in young embryos. At their first appearance, melanophores are randomly distributed over the dorsolateral surfaces and give no indication of barring. Thus, it must follow that any mechanism responsible for barring must come into play after the melanoblasts have migrated from the neural crest and are already *in situ* on the flanks. Xanthophores first appear somewhat after the melanophores; these cells, in contrast to the melanophores, are arranged in irregular bars when they first become visible *in vivo*.

The second alternative was the possibility that regional differences in the ectodermal and mesodermal substrates upon which the colorless pro-pigment cells migrate might in some way impose a pattern of yellow and black bars upon the undifferentiated pigment cells. This possibility also was abandoned on the strength of results from experiments which involved the transplantation of pigment anlagen (neural crest) between barred and non-barred species. The neural crest of young axolotl embryos was first removed, thereby depriving the embryos of their source of axolotl pigment cells. In their places a piece of neural crest from embryos of *Triturus torosus*, a species which does not develop a barred pattern, was grafted. The reciprocal experiment involving the transplantation of axolotl neural crest into crest-free *T. torosus* embryos was also carried out. In both sets of experiments the donor pigment cells behaved in a manner entirely appropriate to their genetic origin; thus, axolotl pigment cells formed bars on the normally non-barred host, and *T. torosus* pigment cells failed to form bars on axolotl hosts. These results indicate that the tissue substrate is probably a neutral factor with regard to the barring phenomenon.

The third alternative was the possibility that the barred pattern might result from interactions between melanoblasts and xanthoblasts of the axolotl and could therefore be intrinsic to the pigment cell population itself. The following experimental evidences tend to support this view. Previous studies (Lehman, 1950 and 1951; Twitty, 1944, and Twitty and Niu, 1948 and 1954) have demonstrated both *in vivo* and *in vitro* that during the early migratory phase of pigment cell differentiation pro-pigment cells exhibit strong negative tissue affinities. The individual cells repel one another and strive for isolation; thus, the direction and distance of migration by a single pro-pigment cell is directly correlated with the number and proximity of its neighboring pro-pigment cells. During the histological differentiation of melanophores, the negative affinity of melanophores for other melanophores diminishes as melanin synthesis nears completion. Of particular interest, however, is the observation (Twitty, 1945) that the negative affinity exhibited between melanophores and xanthophores becomes progressively greater as development proceeds.

When one considers the foregoing facts relative to melanophore and xanthophore behavior, a mechanism which can account for the barring phenomenon suggests itself and can be visualized as taking place in the following steps: 1) Under the pressure of mutual antagonism, melanoblasts leave the neural crest and migrate at random over the flanks. The cells are in this condition when they first become visible *in vivo*. 2) As melanin synthesis proceeds, the melanoblasts (now termed melanophores) become less antagonistic toward one another and their migratory activity ceases. 3) Xanthoblasts which differentiate more slowly than melanoblasts are also assumed to begin their migration later as negative affinities between the xanthoblasts increase in strength. At first, however, the xanthoblasts are impeded by the undifferentiated melanoblasts which have preempted the flank terrain. Later, as the negative affinity between melanophores wanes and the antagonism of xanthophores for melanophores increases, xanthoblasts are finally able to command positions on the flanks. 4) The actual manner in which the xanthophores occupy the flank terrain is visualized as taking place by breaking through the melanophore barrier at those loci which offer the least tissue affinity resistance. The number of break-through points is variable in different individuals and may range from three to seven in the trunk region. 5) After breaching the melanophore barrier at a given point, the strongly antagonistic xanthophores, instead of mixing freely with the flank melanophores, cause them to retreat into compact melanophore bars with the xanthophores then occupying the intervening spaces.

With one exception, all of the above steps in the suggested mechanism for bar formation can be confirmed by direct observations or experimental demonstration. The exception concerns the critical assumption that melanoblasts, in addition to differentiating earlier, also migrate from the neural crest appreciably before the xanthophores. This particular feature should apply only to species such as the axolotl which possess a barred pattern, and should not apply to non-barred species if it is of causal significance in the barring phenomenon. The following tests were devised to discover whether or not this postulated temporal difference between axolotl melanophores and xanthophores does have a basis in fact.

Small pieces of axolotl neural crest were cultured in hanging drop preparations. Approximately 100 cultures were prepared and these were separated into three groups. In one group of cultures the explant was removed after three days while the pro-pigment cells were just beginning to leave the explant (approximately 20 cells per culture). In the second group, the explant was removed on the fourth day (approximately 50 cells per culture), and in the last group the explant was removed on the eighth day when the emigration of pigment cells was essentially complete (approximately 100 cells per culture). All of the cultures were maintained until the distinctive pigments of the individual cells appeared and permitted their classification as to pigment cell type. The results of these experiments were clear-cut and gave positive support to the suggestion that melanophores precede xanthophores in migrating from the neural crest. In the first group of cultures, in which the explant was removed after only three days of isolation,

18 out of 32 cases had no xanthophores and when all cell counts in this series were totaled, xanthophores comprised only 5% and melanophores 95% of all pigment cells. In the second series, in which explants were removed after four days of culture, xanthophores equalled 15% of all pigment cells (*i.e.* a relative increase of 300%). In the last group, in which the explants were removed on the eighth day, xanthophores made up 35% of the total cell count. From this evidence it is quite clear that xanthophores for the most part do begin to migrate from the neural crest appreciably after the first melanoblasts emerge.

As an additional check on the significance of this temporal difference in melanoblast and xanthoblast migration to the barring phenomenon, a comparable series of cultures and explant removals was carried out on a non-barred, closely related species, *Amblystoma punctatum*. In these experiments the relative number of xanthophores in all sets of cultures remained relatively the same irrespective of the age at which the explants were removed. The percentages of xanthophores in the three series were 38%, 41%, and 43% respectively. The lack of a time difference in melanophore and xanthophore migration in this non-barred species increases the likelihood that this factor is indeed of critical importance in the axolotl where such a difference is found in conjunction with barring.

Although definitive proofs are lacking, the foregoing hypothesis is presented as a reasonably accurate representation of the mechanism of bar formation in the axolotl since it is in accord with the events observed in normal development, and it is also in agreement with experimental findings relative to interactions and behavior of axolotl pro-pigment cells.

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A SECOND RECORD OF *MYOTIS SUBULATUS LEIBI* IN NORTH CAROLINA

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The first report of *Myotis subulatus leibi* (Audubon and Bachman) in North Carolina was of a specimen taken at Bat Cave, Rutherford Co. (Adams, Jour. Mamm., **31**(1): 97–98). Southern specimens of this species are seldom taken, and it seems advisable to record the collection of two additional North Carolina individuals. These were taken by J. C. Nicholls, of Murphy, N. C. at a locality 14 mi. N. E. of Andrews, Swain Co. on January 19, 1954, and are numbers 2749 and 2751 in my collection. These bats, a male and a female, were captured in man-made tunnels which had been constructed for the mining of talc; the area is known locally as Talc Mountain. The tunnels in which the bats were found are about 80 feet deep, and both are moist. One individual was taken in flight in the tunnel and the second was secured as it hung from the wall of the tunnel approximately 60 feet inside the tunnel mouth. Since one of these bats was easily disturbed and was taken in flight, it is possible that on this date *Myotis s. leibi* may not have been hibernating. Nicholls indicates that he has previously taken two of these bats in these same tunnels. Other bats found associated with *Myotis s. leibi* at this locality include *Pipistrellus s. subflavus*, *Myotis keeni septentrionalis*, *Eptesicus fuscus fuscus*, and *Plecotus macrotis*. These specimens of *Myotis s. leibi* extend the known range of this bat about 85 miles to the southwest in the southern portion of its range and confirm the montane affinities of *Myotis s. leibi* in the south.

VITAMIN A AND TUMOR GROWTH IN GONADECTOMIZED, METHYLCHOLANTHRENE-INJECTED MICE[1]

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Modifications in body stores of vitamin A, following carcinogen administration, have been described repeatedly in some animals. The present status of the problem is difficult to evaluate, however, because of the variety of animals, carcinogens, dosages, and routes of administration employed. The data ac-

[1] Data obtained from a few animals in some of the groups were included in a thesis submitted to the faculty of Yale University School of Medicine in candidacy for the degree of Doctor of Medicine (1950).

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cumulated can be divided into: (1) those obtained in the presence or absence of induced tumors, or with spontaneous tumor formation; and (2) those illustrating the effect of vitamin A excess or deficiency on tumor growth. A few of the pertinent investigations will be considered briefly below.

1. The presence of induced or spontaneous tumors is accompanied by a decrease in vitamin A stores (Abels, *et al.* 1941, 1942a; Baumann, *et al.* 1941; Goerner and Goerner, 1939a, b; and others). These stores may return to normal values after removal of the tumor (Abels, *et al.* 1942b); neither malnutrition nor malabsorption of the vitamin are causative factors in the results reported. These studies clearly indicate that the presence and growth of the tumor is not essential to the observed changes in values of the vitamin.

2. It has been claimed that vitamin A supplementation inhibits and vitamin A deficiency favors the *in vivo* and *in vitro* growth of induced and spontaneous tumors (Davidson, 1937; Lustig and Wachtel, 1934; Dittmar, 1940); the converse effect has also been reported (Gereb, 1936; Gordonoff and Ludwig, 1937). The inhibitory effect of vitamin A excess and the favorable effect of A-deficiency on growth of transplanted tumors has been claimed (Caspari and Ottensooser, 1930; Dittmar, 1936; Kuh, 1933) and denied (Frankel and Gereb, 1933; Ihara, 1931).

A relationship between vitamin A, gonadal hormones, and carcinogens has been suggested by experimental results obtained by the writer (Truscott, 1944, 1947, 1950, 1953; Rush and Truscott, 1951). It was deemed desirable to attempt to correlate these three types of compounds in a single investigation. The preliminary stages of this study have been reported (Truscott, 1952); the completed study will be presented in this paper.

MATERIALS AND METHODS

Mice of the C_{57} strain were maintained on *ad libitum* feedings of Purina fox chow, and the food intake was measured as described earlier (Truscott, 1953). The mice were weaned on the 23rd–28th day and castrated. The carcinogen-injected group received 0.25 mg. of methylcholanthrene subcutaneously in the approved dosage and method (Shimkin and Andervont, 1941).

Samples of liver (500–800 mg.) were removed, and vitamin A was determined by the antimony trichloride method using a Lumetron and a Klett-Summerson colorimeter. The values are expressed in international units (I.U.) per gram of liver.

RESULTS

Intact mice. With exception of a sharp rise on the 120th postnatal day, the vitamin A values in the non-injected group increased gradually with age; by the end of the experimental period (300 days) the vitamin content was 1362 I.U. per gram of liver (Table 1).

Animals receiving subcutaneous injections of methylcholanthrene showed slightly larger stores of vitamin A, although the shorter survival time of these mice does not permit a completely satisfactory comparison. Marked increases in values were noted between the 120th and 180th days, as contrasted to the smaller amounts in the non-injected group.

TABLE 1

Age (days)	No. of mice		Body weight (gms)		Liver weight (gms)		Vitamin A (I. U./gm)	
	C.*	I.*	C.	I.	C.	I.	C.	I.
1. Hepatic vitamin A in intact mice								
30	12	10	11	12	1.2	1.3	112 ± 23	98 ± 12
60	15	10	20	18	1.3	1.4	292 ± 50	277 ± 42
80	19	7	23	25	1.2	1.8	402 ± 55	321 ± 50
120	13	10	22	25	1.4	1.9	834 ± 65	412 ± 51
180	16	12	26	24	1.7	1.6	520 ± 50	602 ± 48
240	15	0	26	—	1.5	—	685 ± 145	—
300	12	0	30	—	1.9	—	1362 ± 190	—
2. Hepatic vitamin in castrated mice								
30	12	9	11	11	1.0	1.1	208 ± 25	110 ± 24
60	11	8	17	18	1.3	1.3	817 ± 65	754 ± 120
80	12	12	19	18	1.2	1.2	890 ± 60	938 ± 182
120	11	6	21	19	1.2	1.3	1159 ± 110	2225 ± 236
180	11	10	24	21	1.4	1.7	1214 ± 100	2053 ± 250
240	13	0	25	—	1.3	—	1603 ± 135	—
300	8	0	27	—	1.1	—	3173 ± 248	—

* C. = Control; I = injected.

Castrated mice. Hepatic levels of the vitamin in this group are considerably higher than those noted in the intact mice. Throughout the greater part of the experimental period these values are twice as large, and by the 300th day are almost three times those measured in the non-castrated animals.

Subsequent to the 80th postnatal day, hepatic vitamin A stores in injected animals greatly exceed those of non-injected mice. This is not a purely additive effect of castration plus methylcholanthrene. On the 120th day, for example, the vitamin values are 50 per cent greater than could be accounted for by an additive effect.

Induced tumors. The latent period of tumors induced by a single injection of methylcholanthrene was 53–95 days for intact mice, and 47–108 days for castrated mice. The tumor incidence in castrated animals, however, was 17% as contrasted to 33% incidence in the intact group. The wet weights of these tumors averaged 3.2 grams in non-castrated mice, and 0.9 grams in castrates. Although the irregular shape of these tumors precluded accurate size measurements, the weight differences were reflected grossly in the comparative sizes. It was apparent that castration tended to inhibit tumor growth.

Several tumors in intact, injected mice were examined for possible storage of the vitamin. The average values were 26 I.U. per gram, and the total stores averaged 85 I.U. The vitamin A content of these tumors falls within the limits of experimental error, and does not exert an appreciable effect on the total body stores.

The lack of correlation between vitamin A changes and food and caloric intake,

has been confirmed earlier (Truscott, 1953, 1955). A similar lack of correlation with liver and body weight is obvious from the data recorded in Table 1.

Discussion

The tendency toward increased storage of vitamin A in carcinogen-treated mice is in contrast to the reported results of experiments with the rat and the rabbit, in which decreased stores were noted. That multiple intraperitoneal injections of carcinogens do not affect hepatic storage or assimilation of massive oral dose of the vitamin by intact mice, has been noted (Carruthers, 1942). It is interesting, however, that the vitamin A values in the treated mice were larger than those of control animals during certain periods of the investigation; these periods include those reported in our study, during which more apparent differences were observed. Of further interest is that, by the 41st day of treatment, benzpyrene-injected mice, receiving large oral doses of the vitamin, exhibited stores which were almost twice as great as those observed in non-injected animals. In many respects, therefore, the results reported by Carruthers (1942) differ from ours in degree rather than in kind. A more satisfactory evaluation and comparison is impossible due to the lower dosage of carcinogen, smaller number of animals, and different strain of mice employed in the cited investigation.

That factors other than castration or carcinogen-treatment, *per se*, may play a part in producing the results described in this and other studies, is a possibility that should be considered. Although these will be discussed at length in a later publication, a brief consideration of some of these factors would be desirable in the present discussion. 1. Hepatic changes: It has been shown that carcinogen-injection may produce slight, if any, liver damage in animals responding with a marked decrease in stores of the vitamin (Goerner and Goerner, 1939a, b, 1943; Marron, 1941). It has been indicated, furthermore, that the present study fails to demonstrate a correlation between liver size and vitamin A values in castrated and/or carcinogen-treated mice. 2. Altered food intake: Neither the above investigation, nor others (Goerner, 1938; Truscott, 1953), offers an explanation for altered vitamin stores in these animals on the basis of differences in daily food intake. The very large stores of vitamin observed in castrated mice are of particular interest in view of reports of a decrease in food intake (Tang, 1941) and in efficiency of food utilization (Palmer, *et al.* 1946) in these animals. 3. Altered body growth: Lack of correlation of this factor with changes in vitamin A stores under experimental conditions has been demonstrated (Baumann, *et al.* 1941), and is confirmed in the present investigation. 4. Altered metabolism of the vitamin: changes in absorption, utilization, or destruction of the vitamin do not explain satisfactorily the results obtained (Glover, *et al.* 1947; Popper and Volk, 1948). Normal pregnancies in the carcinogen-treated mice, furthermore, suggest that the increased hepatic storage of the vitamin is not due to a marked decrease in its utilization. 5. Influence of the solvent: The described side-effects of sesame oil, the solvent for the carcinogens used in these experiments (Matthews, *et al.* 1947; Tobin, 1944), are apparently without influence on the changes in vitamin A values described here.

Results of the present investigation may have an important bearing on the concept of a relationship between vitamin A, carcinogens and steroid hormones (Truscott, 1944, 1954). A possible physiological and chemical basis for the suggested association receives extended discussion in a paper to be published later (Truscott, 1955). At the present time, however, it should be recognized that the complex associations of vitamin A remain without a comprehensive and satisfactory explanation.

SUMMARY

1. Increased stores of hepatic vitamin A are present in mice of the C_{57} strain receiving injections of methylcholanthrene.

2. Castrated mice contain greatly increased hepatic stores of the vitamin; the latter are greater in mice given a single subcutaneous injection of the carcinogen following castration.

3. The liver weights of castrated mice, injected or non-injected, are subnormal; those of intact mice receiving a single subcutaneous injection are greater than normal.

4. Tumor size, weight, rate of growth and incidence appear to be decreased in castrated mice.

5. Neither food intake nor body growth can be correlated with the body stores of vitamin A.

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COMMENTS ON THE TEACHING OF TRIGONOMETRY*

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Trigonometry originated with the problems of surveying and navigation, but it has subsequently developed continually and spread into numerous fields of mathematical and scientific analysis.

Without minimizing the importance of the computational theme, the work which follows is an attempt to streamline the topic relating specifically to the derivation of formulas for solving triangles. As a preliminary background the student should become thoroughly familiar with the projection method and the concept of open and closed traverses.

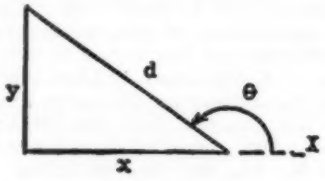

FIG. 1

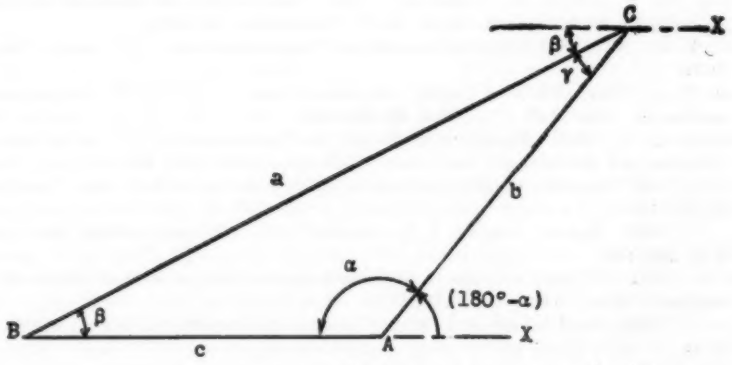

FIG. 2

Treating the triangle ABC as a closed traverse, and noting that the horizontal and vertical projections (Fig. 1) are $x = d \cos \theta$, $y = d \sin \theta$; where d is length

* Presented before the Mathematics Section of the 51st Annual Meeting of the N. C. Academy of Science at Greenville, May 7-8, 1954.

of line and Θ is the angle it makes with the X-axis; we proceed as follows [see Fig. 2]:

	Distance	Angle	x	y
AC	b	$180° - \alpha$	$-b \cos \alpha$	$b \sin \alpha$
CB	a	$180° + \beta$	$-a \cos \beta$	$-a \sin \beta$
BA	c	$0°$	c	0
		Sum =	0	0

From $\Sigma y = 0$;

(1) $$a \sin \beta = b \sin \alpha \qquad \text{[Law of Sines]}$$

From $\Sigma x = 0$;

(2) $$a \cos \beta = c - b \cos \alpha \qquad \text{[Projection Law]}$$

Squaring Eqs. (1) and (2), then adding and noting that $\sin^2 \alpha + \cos^2 \alpha = 1$ [also $\sin^2 \beta + \cos^2 \beta = 1$], we get

(3) $$a^2 = b + c^2 - 2bc \cos \alpha \qquad \text{[Law of Cosines]}$$

Dividing Eq. (1) by Eq. (2), and noting that $\tan \beta = \dfrac{\sin \beta}{\cos \beta}$, we get

(4) $$\tan \beta = \frac{b \sin \alpha}{c - b \cos \alpha} \qquad \text{[Tangent Formula]}$$

Interchanging the letters b and c, we have

(5) $$\tan \gamma = \frac{c \sin \alpha}{b - c \cos \alpha} \qquad \text{[Tangent Formula]}$$

Eq. (4) [or Eq. (5)] is offered as a substitute for the familiar law of tangents, which, in the opinion of the writer, is objectionable for two reasons, (1) its proof is not simple, (2) it deals with the *difference* of two numbers, and therefore might not be sufficiently accurate when the given numbers are nearly equal.

Given b, c, and α (2 sides and the included angle) Eq. (4) is preferable if $c > b$. Likewise, Eq. (5) is preferable if $b > c$; it being desirable (but not necessary) that the denominator be positive and the computed angle less than 90°.

In applying these or any other analogous formulas (obtained by a cyclic change of letters), the first part to be calculated is the angle opposite the shorter of the two given sides. To complete the solution, the third side is found by means of the law of cosines. Thus if $c > b$, we use the following pair of formulas:

$$\text{(a)} \quad \tan \beta = \frac{b \sin \alpha}{c - b \cos \alpha}; \qquad \text{(b)} \quad a = \frac{b \sin \alpha}{\sin \beta}.$$

It is seen that the numerators of Eqs. (a) and (b) are identical. In using log-

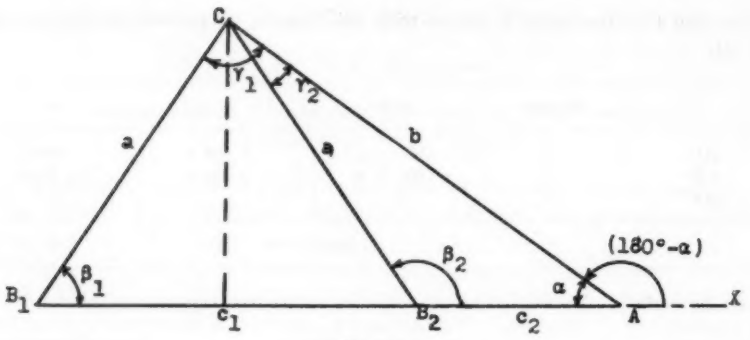

FIG. 3

arithms, the anti-log of the product $b \cos \alpha$ is to be found separately, otherwise the solution by logs. follows readily.

The use of these formulas is in effect a projection on the *longer* of the two given sides. Hence to obtain an independent and *valid* check, we must project on the *shorter* of the two given sides. Thus, in this case the check would be

$$b = c \cos \alpha + a \cos \gamma$$

Given α, a, and b [when $\alpha < 90°$ and $a < b$], Fig. 3 illustrates clearly the ambiguous case. Applying Eq. (1), we have

$$\sin \beta_1 = \frac{b \sin \alpha}{a} \tag{6}$$

Since $\beta_2 = 180° - \beta_1$, the solutions of the two triangles may now be completed by means of well established methods.

The foregoing formulas represent all that are actually needed for the complete solution of oblique triangles.

Since the law of cosines is not conveniently adapted when large numbers are encountered, the so called half-angle formulas are given in textbooks on trigonometry to facilitate the use of logarithms for this purpose. However, time (derivation time) is saved by dealing only with the sine and omitting the tangent. Thus

$$\sin^2 \tfrac{1}{2}\alpha = \left(\frac{s-b}{b}\right)\left(\frac{s-c}{c}\right); \qquad \sin^2 \tfrac{1}{2}\beta = \left(\frac{s-a}{a}\right)\left(\frac{s-c}{c}\right);$$

$$\sin^2 \tfrac{1}{2}\gamma = \left(\frac{s-a}{a}\right)\left(\frac{s-b}{b}\right).$$

THE UTILIZATION OF CARBON AND NITROGEN COMPOUNDS BY *PHYTOPHTHORA PARASITICA* DASTUR VAR. *NICOTIANAE* (BREDA DE HAAN) TUCKER[1]

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INTRODUCTION

Information concerning the nutrition of species of *Phytophthora* is meagre. The ability of *P. parasitica* Dastur, *P. palmivora* Butler, and *P. cactorum* (L. and C.) Schroet. to utilize various carbohydrates was tested by Volkonsky (1934). Acid production was the criterion of utilization which he used. Leonian and Lilly (1938, 1940) determined that thiamine affects the utilization of various compounds by *P. erythroseptica* Pethyb. More recently, Mehrotra (1951) studied the carbon requirements of ten Phytophthoras, including the black shank organism. The present report concerns the utilization of various carbohydrates, organic acids, alcohols, and amino acids as single carbon sources, and utilization of amino acids and other compounds as sole sources of nitrogen by an isolate of the tobacco pathogen.

METHODS AND MATERIALS

The isolate tested was obtained from the North Carolina Tobacco Experiment Station, Oxford, North Carolina. Stock cultures were maintained on potato-dextrose agar at room temperature.

A basal medium of the following composition was prepared: KCl, 0.5 g.; $MgSO_4 \cdot 7H_2O$, 0.5 g.; $NaNO_3$, 3.86 g.; Sucrose, 30 g.; Thiamine, 0.001 g.; and distilled water, 1000 ml. The nitrogen and carbon sources were substituted singly for $NaNO_3$ and sucrose in the basal medium in amounts necessary to maintain the carbon and nitrogen at the level of the basal medium, i.e., 400 mg. carbon and 63.6 mg. nitrogen per 100 ml. of solution.

All media were adjusted to pH 6.0 and were buffered at this value. A citric acid-Na_2HPO_4 buffer pair was used in the studies involving nitrogen sources, and a NaOH-KH_2PO_4 buffer was used in the studies involving carbon sources. The necessary phosphate was thus supplied in the buffer. In the preparation of media chemicals for 50 ml. of solution were dissolved in 30 ml. of distilled water and 20 ml. of buffer solution were added. The cultures were grown in 50 ml. of media in 250 ml. Erlenmeyer flasks. The Pyrex flasks used were cleaned in sulfuric acid-potassium dichromate cleaning solution and were thoroughly rinsed in distilled water. All media were autoclaved at 15 pounds pressure for 15 minutes, the carbohydrates being autoclaved separately and added to the medium aseptically.

The flasks of media were inoculated with as small an inoculum as possible and were maintained for 30 days at room temperature. The amount of growth

[1] The cost of publication of this and the following eight papers was borne by Duke University.

was measured by determining the dry weight of the mycelial mat from each flask. At the end of the growth period the mats were rinsed in distilled water, dried at 60°C. for 24 hours on tared filter paper, and stored in a $CaCl_2$ desiccator until weighed. All tests were replicated three times and the results arranged in four classes on the basis of weight of mycelium. The class ranges were determined arbitrarily by dividing the difference between the minimum (zero) and maximum weight of the mats into four classes.

RESULTS

CARBON SOURCES: Twenty-five carbohydrates, organic acids, and alcohols were tested as single sources of carbon for growth of the fungus. Since some fungi will utilize amino acids as a source of carbon (Gottlieb, 1946), amino acids also were tested as sources of carbon for the tobacco pathogen. The compounds tested were added individually to the basal medium from which sucrose was omitted. The results are presented in Table I.

As may be seen, the polysaccharides, dextrin and starch, proved to be the best carbon sources tested. The next best carbon sources were the disaccharides,

TABLE I

Growth of Phytophthora parasitica *var.* nicotianae *in a basal medium in which various carbohydrates, organic acids, alcohols and amino acids were added as single sources of carbon*

Substrate	Amount of growth	Substrate	Amount of growth
Arabinose	0	Glycine	I
Xylose	I	beta-Alanine	I
Levulose	I	l-Glutamic acid	I
Galactose	I	l-Cystine	0
Mannose	I	l-Cysteine·HCl	0
Sucrose	II	l-Leucine	I
Maltose	II	l-Lysine·HCl	I
Cellobiose	II	l-Histidine·HCl	I
Lactose	0	l-Arginine·HCl	I
Raffinose	0	l-Tryptophane	0
Dextrin	IIII	l-Tyrosine	I
Starch	IIII	l-Proline	I
α-Methyl glucoside	I	l-Hyroxyproline	0
Acetic acid	0	dl-Alanine	I
Succinic acid	0	dl-Aspartic acid	I
Tartaric acid	0	dl-Serine	I
Oxalic acid	0	dl-Threonine	I
Citric acid	0	dl-Valine	0
Malic acid	0	dl-Isoleucine	0
Fumaric acid	0	dl-Norleucine	I
Glycerol	I	dl-Phenylalanine	0
Mannitol	0	dl-Ornithine·HCl	I
Dulcitol	0	dl-Methionine	0
Sorbitol	0	l-Asparagine	II
Adonitol	0	Control (no carbon)	0

TABLE II

Growth of Phytophthora parasitica *var.* nicotianae *in a basal medium in which various nitrogen-containing compounds were substituted for* $NaNO_3$

Substrate	Amount of growth	Substrate	Amount of growth
Glycine	I	dl-Aspartic acid	I
beta-Alanine	I	dl-Serine	II
l-Glutamic acid	II	dl-Threonine	III
l-Cystine	I	dl-Valine	I
l-Cysteine·HCl	0	dl-Isoleucine	0
l-Leucine	I	dl-Norleucine	0
l-Lysine·HCl	I	dl-Phenylalanine	0
l-Histidine·HCl	IIII	dl-Ornithine·HCl	IIII
l-Arginine·HCl	II	dl-Methionine	0
l-Tryptophane	0	l-Asparagine	I
l-Tyrosine	I	$NaNO_3$	I
l-Proline	II	$(NH_4)_2HPO_4$	I
l-Hydroxyproline	I	NH_4NO_3	I
dl-Alanine	I	Urea	0

sucrose, maltose, and cellobiose. Arabinose, lactose, and raffinose were not utilized. Organic acids, and the alcohols, except glycerol, were not utilized. Fifteen amino acids were utilized, but all were poor sources of carbon. Asparagine was a better carbon source than any of the other amino acids. The sulfur-containing amino acids were not utilized. Other animo acids which were not utilized included tryptophane, hydroxyproline, valine, isoleucine, and phenylalanine.

To determine whether the alcohols were unavailable or were inhibitory, the alcohols were tested as before, but the media were supplemented with asparagine. Even when asparagine was added, glycerol was the only alcohol which supported growth, the amount of growth being approximately the same as in a medium containing only asparagine as a carbon source. It appears that the alcohols were inhibitory to growth of the fungus.

NITROGEN SOURCES: Twenty-four amino acids, $NaNO_3$, $(NH_4)_2HPO_4$, NH_4NO_3 and urea were tested as sole sources of nitrogen for growth. The compounds tested were added singly to the basal medium from which $NaNO_3$ was omitted. The results are presented in Table II.

Among the compounds tested, histidine, ornithine and threonine were superior to all others as sources of nitrogen. Glutamic acid, arginine, proline, and serine were the next best sources of nitrogen. None of the remaining compounds was a good source of nitrogen for the tobacco *Phytopthhora*. Urea and the amino acids cysteine, tryptophane, isoleucine, norleucine, phenylalanine, and methionine were not utilized by the fungus as nitrogen sources.

DISCUSSION

The results of the utilization of carbohydrates and alcohols by this strain of the black shank fungus are in surprisingly close agreement with the results obtained by Volkonsky (1934) on three isolates of *P. parasitica* and single strains

of *P. palmivora* and *P. cactorum*. He found that glycerol, erythritol, sorbitol, mannitol, dulcitol, arabinose, xylose, rhamnose, galactose, lactose, and raffinose were fermented slowly or not at all, whereas glucose, levulose, maltose, saccharose, dextrin, and starch were fermented rapidly. Levulose was fermented rapidly by Volkonsky's strains of *Phytophthora*, but it was a poor source of carbon for the tobacco fungus; otherwise the results of the two studies are in general accord. One of Volkonsky's strains of *P. parasitica* varied as much from the other two strains as the two differed from the other species.

The ten Phytophthoras tested by Mehrotra (1951) utilized all the carbohydrates and alcohols tested as carbon sources. With a few exceptions, Mehrotra used the same carbohydrates and alcohols for carbon sources as were used by Volkonsky and the author in the present investigations. In general, the compounds which were not utilized or were poorly utilized as carbon sources in the present study proved to be the poorer carbon sources in the investigations by Mehrotra. There were some differences, however, between the present study and Mehrotra's study. The basal media used in the two studies contained different salts and different nitrogen sources. In the present study the media were buffered, but no buffer was used by Mehrotra. These differences conceivably could affect the final results. It would seem that the emphasis should be placed upon the similarities of the results of these studies, rather than upon the differences.

Leonian and Lilly (1938) reported that *P. erythroseptica* utilized arginine, aspartic acid, glutamic acid, glycine, alanine, histidine, proline, and serine, and that isoleucine, tryptophane, and valine were not so well utilized as nitrogen sources. Thiamine was required for growth, regardless of the nitrogen source. Five of the amino acids used by *P. erythroseptica* were among the best nitrogen sources for the tobacco fungus. As nitrogen sources, isoleucine and tryptophane were not utilized by the tobacco fungus, however, and aspartic acid, glycine, alanine, and valine supported only moderate growth. Leonian and Lilly (1940) have shown that balance among the sugar, the amino acids and thiamine present controls the amount of mycelium produced by *P. erythroseptica*. Comparison of the results of studies on nitrogen sources cannot be made, therefore, even if the same species is used, if this balance among components of the medium is not duplicated. Failure of a fungus to grow on a medium containing a single amino acid as a nitrogen source should not be taken as evidence for non-availability of the particular amino acid unless supported by more extensive studies having a wider range of nutritional conditions.

Information obtained in this study has been used to prepare a suitable chemically defined medium for growth of *P. parasitica* var. *nicotianae*, using soluble starch as the carbon source and glutamic acid as a nitrogen source.

SUMMARY

A group of carbohydrates, organic acids, alcohols, and amino acids were tested singly as carbon sources for growth of *Phytophthora parasitica* var. *nicotianae* in media containing $NaNO_3$ as the nitrogen source. Dextrin and starch proved to be the best sources of carbon for the fungus, followed by sucrose,

maltose, and cellobiose. No organic acid tested was utilized by the fungus. Glycerol was the only alcohol utilized, the other alcohols tested being inhibitory to growth of the fungus. Asparagine was utilized more rapidly than any amino acid as a carbon source, although 15 amino acids were utilized poorly. There was a general similarity of results on utilization of carbon sources by the tobacco fungus to those found with strains of *Phytophthora* studied by two earlier workers.

In media containing sucrose as the source of carbon, a number of amino acids and a few other nitrogen-containing compounds were tested as nitrogen sources. The best nitrogen sources were found to be ornithine, histidine, threonine, serine, proline, arginine, and glutamic acid. Six amino acids and urea were not utilized as nitrogen sources. Inorganic nitrogen was poorly utilized.

As a result of this study, a synthetic medium containing soluble starch as the carbon source and glutamic acid as the nitrogen source was devised for studies with *P. parasitica* var. *nicotianae*.

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SPORANGIUM FORMATION BY *PHYTOPHTHORA PARASITICA* DASTUR VAR. *NICOTIANAE* (BREDA DE HAAN) TUCKER

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INTRODUCTION

Numerous factors are known to be involved as interdependent variables in stimulating the fruiting response among fungi. Moreover, the fundamental biochemical mechanisms by which these factors operate remain largely unknown or only partly understood.

Certain soil-inhabiting phycomycetes, as *Phytophthora cinnamomi* Rands, have been noted to sporulate in soil leachate (Mehrlich, 1935). The factors present in such solutions which are involved in sporulation by these organisms have not been elucidated however. The writer noted that a soil agar supported abundant sporulation of the black shank fungus, *Phytophthora parasiticia* var. *nicotianae*, although vegetative growth was sparse on this medium. It was

further noted that a water extract of soil was even more effective in inducing sporulation. A study was therefore undertaken to determine the nature of this sporulation-inducing property of soil. The term sporulation is hereinafter restricted to mean the production of sporangia. The fruiting response of this fungus to soil extract was prompt, sporulation beginning at times within six hours after transfer of mycelium to the extract, and within 24 hours sporulation was usually abundant. It thus appeared that perhaps a specific substance present in soil was responsible for the abrupt change from vegetative growth to sporulation.

METHODS

The isolate of the black shank fungus used in this study was obtained through courtesy of the Tobacco Experiment Station, Oxford, North Carolina. Although 23 isolates were tested for sporulation in soil extract and only one failed to sporulate, a single isolate was used consistently throughout the study. The cultures were maintained on potato-dextrose agar, and the inoculum was grown at room temperature in 125 ml. Erlenmeyer flasks containing 50 ml. of potato-dextrose broth. The broth was prepared by boiling 200 g. of potatoes in 1000 ml. of water for 30 minutes and adding 30 g. of dextrose to the filtrate after removing the solids by filtration through several layers of cheesecloth.

The oat broth used in the preliminary survey was prepared from 50 g. of powdered oat grains boiled for one hour in 1000 ml. of distilled water. The composition of the nutrient solution, fractions of which were tested in the preliminary survey, was as follows: KCl, 0.5 g.; KH_2PO_4, 1.0 g.; $MgSO_4 \cdot 7H_2O$, 0.5 g.; soluble starch, 9.0 g.; histidine, 1.07 g.; H_3BO_3, 1.0 mg.; $MnSO_4$, 1.0 mg.; $ZnCl_2$, 1.0 mg.; $CuSO_4 \cdot 5H_2O$, 1.0 mg.; $FeCl_3$, 1.0 mg.; $(NH_4)_6Mo_7O_4 \cdot 4H_2O$, 1.0 mg.; riboflavin, 100 μg.; pyridoxine HCl, 200 μg.; thiamine HCl, 200 μg.; niacine, 200 μg.; calcium pantothenate, 200 μg.; i-inositol, 1000 μg.; biotin, 10 μg.; and distilled water, 1000 ml.

The soil extracts were prepared as follows: 300 g. of air-dry soil were boiled for one hour in 1000 ml. of water. The solids were removed by vacuum filtration and the resulting solution was used undiluted. To study the effect of destruction of organic matter in soil, duplicate 100 g. samples of air-dry soil were prepared. One of each pair of samples was burned in an electric muffle furnace for three hours at 600 to 650°C, after which the paired samples were extracted with water, as described above.

In treating solutions with charcoal, 20 g. of Mallinckrodt's decolorizing charcoal were added to 1000 ml. of solution, after which the mixture was stirred mechanically for one hour. The charcoal was removed by vacuum filtration and the filtrate was sterilized in the autoclave before testing. In treating charcoal with acid and alkali, 0.01 N HCl and 0.01 N NaOH were used as the eluting liquid and the eluates were adjusted to pH 6.5–7.0. In treating distilled-water eluates from charcoal with ion adsorption resins, Dowex-50 and Dow-1* were used as the cation- and anion-exchange resin respectively. The eluates were treated by

* Dow Chemical Co.

mechanically stirring 10 g. of the appropriate resin in 100 ml. of the eluate for two hours, after which the resins were removed by filtration.

The method of Steinberg (1935) was employed for the purification of solutions in the experiment to determine the effect of heavy-metal contaminants. The method consists of adding 15 g. of $CaCO_3$ to each 1000 ml. of solution, autoclaving for 15 minutes at 15 pounds pressure, and then removing the precipitate by filtration. The $CaCO_3$ serves to alkalize the solution, causing the precipitation of insoluble calcium salts and the coprecipitation of heavy metal salts.

The various media and test solutions were autoclaved at 15 pounds pressure for 15 minutes and were used in 25 to 30 ml. amounts in 125 ml. Erlenmeyer flasks. All glassware was Pyrex and was cleaned in potassium dichromate-sulfuric acid cleaning solution and rinsed thoroughly in distilled water. All pH determinations were made with a Beckman pH meter. The inoculum was grown for from 3 to 5 days, transferred aseptically to sterile distilled water and thence to the test solution. At the end of the test period, ordinarily 24 hours at room temperature, a sample of the mycelium was removed from each flask and examined under low power of the microscope. All tests were conducted in triplicate. The amount of sporangium formation is indicated in five classes, 0, + (meagre sporulation), ++ (fair sporulation) and +++ and ++++ (abundant sporulation).

RESULTS

A preliminary survey was made to test the sporulation-promoting capacity of various solutions, including tap water, distilled water, soil extract, oat broth dilutions from full strength to 0.01% full strength, and the various groups of

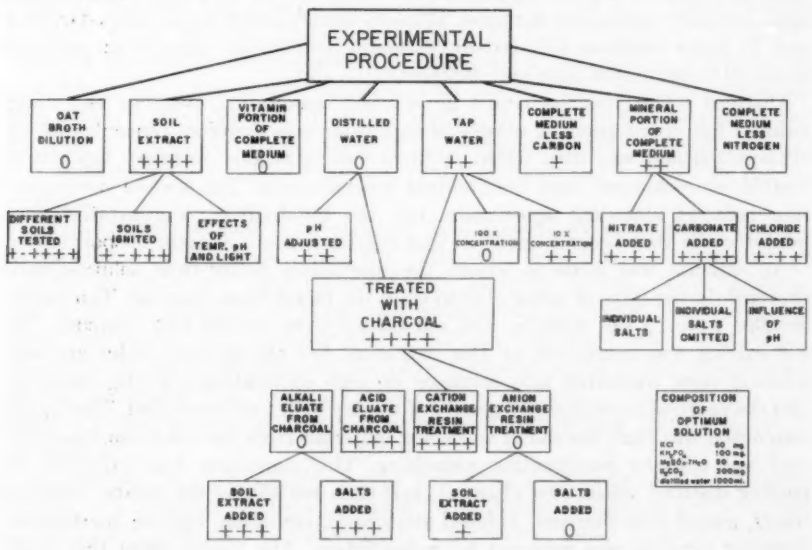

FIG. 1

components of a complete nutrient solution. As indicated in the flow diagram of procedure (Fig. 1), this survey yielded evidence that several solutions are capable of inducing sporulation and suggested further lines of investigation. Soil filtrate induced abundant sporulation, tap water fair sporulation, and the mineral solution fair sporulation. Meagre sporulation or no sporulation was obtained in the other solutions tested. Obviously soil extract provided the most favorable medium for sporulation which was tested.

Filtrates (extracts) of six soils were prepared, all of which induced fair to abundant sporulation. Extracts were then prepared from matched samples of six ignited and non-ignited soils, by the method earlier described, and were tested for sporulation promotion. In general, extracts of ignited soil were as effective in inducing sporulation as were the extracts of the non-ignited soils. The results were variable but conclusively showed that the sporulation factor in soil is inorganic in nature. The temperature range for sporulation of the fungus in soil extract was studied by incubating mycelium of the fungus in soil extract over the range of temperature from 6°C. to 37°C., in steps of 5°C. The cardinal temperatures were found to be approximately 15°C., 20–25°C. and 30°C. The influence of pH on sporulation of the fungus in soil extract was tested by incubating mycelium for 24 hours in soil filtrates, the pH being adjusted in steps of 0.4 pH over the range of pH 5.4 to pH 11.0. The amount of sporulation varied at different pH values but no optimum pH was evident and at least fair sporulation was obtained at all pH values in this range. To determine the influence of light on sporulation, the fungus was grown under continuous illumination, in total darkness, and in alternate light and darkness. Mycelium from each of these treatments was transferred to soil extract and subjected to 24 hours continuous light, 24 hours continuous darkness, 12 hours light followed by 12 hours darkness and 12 hours darkness followed by 12 hours light. Good sporulation occurred under all conditions of light and darkness.

Distilled water was ineffective in inducing sporulation, whereas tap water induced fair sporulation. A sample of tap water was therefore concentrated by evaporation and was then tested in three concentrations; ordinary tap water, tenfold concentration, and hundredfold concentration. The tenfold concentration induced abundant sporulation but the hundredfold concentration was inhibitory. It thus became apparent that concentration is a factor in sporulation.

An attempt was made to adsorb the sporulation factor from solution onto charcoal, in the hope of being able to elute the factor from charcoal. Tap water, distilled water, a salt solution, and soil extract were treated with charcoal. The soil extract was unaffected by this treatment but the distilled water and salt solution were converted into solutions of high sporulation-inducing capacity, and the sporulation-inducing capacity of the tap water was increased. The logical conclusion was that, instead of adsorbing materials from the solutions, the charcoal was actually contributing something. This conclusion was validated by passing distilled water over charcoal, and then redistilling the eluate. Distilled water, passed over charcoal, induced abundant sporulation, but the sporulation-inducing capacity was removed by redistillation. The residue from this distil-

TABLE I

Sporangium formation by P. parasitica *var.* nicotianae *in acid and alkali eluates from charcoal*

Test solution	pH	Sporangium formation after 24 hours
Distilled water control	5.6	0
N/100 HCl, pH adjusted with N/1 NaOH (control)	6.8	++
N/100 NaOH, pH adjusted with N/1 HCl (control)	5.7	++
Distilled water eluate from charcoal	6.0	++++
N/100 HCl eluate from charcoal, pH adjusted with N/1 NaOH	6.8	++++
N/100 NaOH eluate from charcoal, pH adjusted with N/1 HCl	6.4	0
Distilled water eluate from the charcoal which was previously treated with N/100 HCl	7.1	+++
Distilled water eluate from the charcoal which was previously treated with N/100 NaOH	7.6	0

lation, when taken up in distilled water, constituted an effective sporulation-promoting solution.

Dilute (0.01 N) HCl and NaOH were used to wash charcoal. The eluates were adjusted to a pH near neutrality before testing. Controls were provided by solutions containing NaOH and HCl only. The results are presented in Table I.

It is apparent that dilute HCl removes from charcoal material which induces sporulation by the fungus. Abundant sporulation occurred in the water used to wash the charcoal which had been treated previously with acid. Dilute NaOH, however, either removes inhibitory materials or fails to remove sporulation-inducing materials from the charcoal. Distilled water did not elute a sporulation factor from the alkali-treated charcoal. This suggests that inhibitory materials were eluted in the dilute NaOH. Such inhibiton was found to be overcome by the addition of soil extract to the alkali eluate, suggesting that the inhibition was due to improper balance of materials in the solution, and not to any specific substance.

Distilled water eluates from charcoal were treated with anion- and cation-exchange resins. Treatment with the cation-exchange resin did not alter or slightly increased the sporulation-inducing capacity of the eluate, whereas treatment with the anion-exchange resin rendered the eluate inhibitory to sporulation. Inhibition was overcome within five days, however, when soil extract was added to the eluate. Sporulation never occurred in the eluate treated with the anion-exchange resin without the addition of soil extract. This indicates that either a proper balance of anions or some specific anion is a limiting factor in sporulation.

As indicated, the preliminary survey had yielded evidence that the mineral portion of a complete medium provided for induction of sporulation, although the sporulation was only fair in amount. A mixture of minor elements failed to produce a fruiting response. Exploratory experiments, testing a three-salt

solution of the following composition; KCl, 0.5 g.; KH_2PO_4, 1.0 g.; $MgSO_4 \cdot 7H_2O$, 0.5 g., and distilled water, 1000 ml., supplemented by various chloride, nitrate, phosphate and carbonate salts, yielded erratic results. Various combinations of salts produced solutions of slight sporulation-inducing capacity. The pH of the medium proved to be a limiting factor to sporulation in defined salt solutions, being effective between pH 7 and pH 10. The addition of a carbonate salt to the three salt solution provided the best defined medium for sporulation. The only defined medium capable of inducing sporulation in amount equal to the sporulation occurring in soil extract had the following composition: KCl, 50 mg.; KH_2PO_4, 100 mg.; $MgSO_4 \cdot 7H_2O$, 50 mg.; K_2CO_3, 300 mg.; and distilled water, 1000 ml. A solution of such components and such dilution induced abundant sporulation when unadjusted at pH 9.0 and when adjusted at pH 7.4. Using this solution as a basal medium, each component was omitted singly from the complete medium and each was tested individually as a single-salt solution. The results are presented in Table II.

It may be seen that the complete solution, at pH 7.2, is superior to any fraction of the complete solution, regardless of the pH. K_2CO_3 alone is effective in inducing sporulation in a single-salt solution, but only at an alkaline pH. How pH and the materials in the medium interact is not clear. Abundant sporulation occurs in the complete solution at pH 7.2, but sporulation is meagre in the same solution at pH 6.2. At pH 6.8 distilled water supports no sporulation but fair sporulation occurs at pH 8.7 in distilled water. Similar relations exist for other defined solutions. Soil extracts, however, are relatively insensitive to pH in promoting sporulation.

To determine whether heavy-metal contaminants of the salts exerted any influence upon sporulation, the solutions were purified by the method of Steinberg (1935). A complete solution treated by this method was as effective in

TABLE II

Sporangium formation by P. parasitica *var.* nicotianae *in various portions of a four-salt solution, in the complete solution and in distilled water*

Test solution	Initial pH	Sporangium formation after 36 hours	Final pH
Complete	7.2	+++	7.1
Complete, pH adjusted	6.2	+	6.7
minus KCl	7.4	++	6.8
minus $MgSO_4 \cdot 7H_2O$	7.6	++	7.0
minus KH_2PO_4	8.1	++	6.8
minus K_2CO_3	6.4	++	6.8
minus K_2CO_3, pH adjusted	8.9	++	7.0
only KCl	6.1	0	6.1
only KH_2PO_4	6.0	0	6.5
only K_2CO_3	8.4	++	6.9
only K_2CO_3, pH adjusted	6.1	0	6.8
distilled water	6.8	0	4.8
distilled water, pH adjusted	8.7	++	7.1

inducing sporulation as was soil extract, indicating that whatever heavy-metal trace elements were present as contaminants of the salts may be disregarded. If such trace elements are necessary for sporulation they must be present in sufficient quantity in the inoculum.

DISCUSSION

Sporangium formation by the black shank fungus may be induced by inorganic materials found in soil, tap water, or charcoal. Sporulation is in part dependent upon concentration of the materials, as shown by the experiment in which tap water was concentrated by evaporation. It is probably dependent in part upon a proper balance of ions also. Sporulation-inducing materials may be eluted from charcoal by distilled water or by dilute HCl but not by dilute NaOH. On the basis of solubility, a greater number of materials would be expected to be eluted by acid than by alkali. This probably accounts for the lack of balance in the alkali eluate necessary to promote sporulation. Treatment of a water eluate from charcoal with a cation-exchange resin did not reduce the sporulation-promoting ability of the eluate, whereas treatment with an anion-exchange resin rendered the eluate inhibitory. Presumably the latter treatment would result in a high concentration of chloride ions and thus provide an unfavorable balance of ions. A high concentration of sodium ions would be expected from the treatment with the cation-exchange resin, yet sporulation is not inhibited by this treatment. Perhaps the balance among anions is more important than the balance among cations.

A sporulation-inducing medium of known composition, if made to contain KCl, KH_2PO_4 and $MgSO_4 \cdot 7H_2O$, induces meagre sporulation, but if supplemented with any of various carbonate, nitrate, phosphate or chloride salts it supports increased sporulation. Carbonate salts are particularly effective in this respect. Variable results were obtained with different combinations of these salts, thus emphasizing the importance of the proper balance and concentration of ions in these solutions.

In the light of the sporulation response by the black shank fungus in the presence of carbonates, it may be indicated that the biochemical transformations leading to sporulation may be triggered by carbonates. This interpretation is supported by findings by Emerson and Cantino (1948), in which *Blastoclodia pringsheimii*, when grown under 99.5% carbon dioxide or in media containing carbonates, produced resistant sporangia in large numbers. Further substantiation comes from results with *Blastocladiella emersonii* Cant. and Hyatt. Cantino (1951, 1952) noted that bicarbonate in the immediate environment determines that spores derived from resistant sporangia grow to become thalli bearing thick-walled resistant sporangia. In the absence of carbonates, however, such spores grow to become thalli bearing thin-walled sporangia (gametangia).

The effect of pH in conjunction with the proper balance and concentration of salts cannot be disregarded or separated from the effect of the other factors. Distilled water, adjusted to pH 8 to pH 9, will induce a moderate amount of sporulation, whereas the most suitable defined solution is ineffective below pH 6.

Soil extract is effective in promoting sporulation over a wide range of hydrogen-ion concentration. In general, a pH of the medium above 7.9 is more favorable for sporulation than an acid pH. A favorable pH of the medium and a favorable balance and concentration of inorganic ions are requisite to eliciting the fruiting response by the fungus. The ultimate mode of interaction of these factors remains unknown.

Having considered the importance of soil in the fruiting of the black shank fungus, the possible significance of soil in the developmental cycle of the fungus should be considered. Soil is a medium in which the black shank *Phytophthora* and other members of the genus can grow and be dispersed. Although isolations of *Phytophthora* are readily made from diseased plants, at least one member of the genus, *P. cinnamomi*, has been isolated directly from the soil where it was not associated with any diseased plant (Campbell, 1951). Blackwell (1944) suggested that many of the Phytophthoras exist in soil for long periods as water molds until carried to susceptible hosts by water.

How the black shank fungus and other Phytophthoras come to be present in new regions is problematic. Since the soil is a suitable medium for these fungi it may be considered as a possible primary habitat, in which case the pathogenic mode of existence may be considered as a secondary and fortuitous mode of existence. Tobacco is considered to be of relatively recent hybrid origin and numerous changes have arisen in the plant by intensive breeding. Perhaps the potential pathogen has existed as an innocuous soil organism until circumstances of environment, variation in the host, and variation in the fungus have coincided to make possible the pathogenic relationship. Recently deBruyn (1951) demonstrated that passage through resistant hybrids of tomato and potato induced changes in pathogenicity of certain strains of *P. infestans*. It seems reasonable to assume the possibility of similar reactions occurring in the black shank fungus.

Members of the genus *Phytophthora* are notorious as destructive plant pathogens. The genus is equally notorious as a taxonomic enigma, and no satisfactory method of delimiting species has been devised. Furthermore, the various named species have frequently been found to cross, with the production of oospores (Thomas *et al.* 1947, 1948). Many of the species may be only strains or varieties. By crossing, new variants in pathogenicity may arise. Such variation, together with non-sexually produced variation, as is known to exist in the black shank fungus, may be an important factor in the breakdown of resistance in tobacco bred for resistance to attack by this organism. Thus the assumption of a high order of variation in the fungus causing black shank and the assumption that the fungus is primarily a soil organism becomes possible. The verification or refutation of these assumptions constitutes an important area of research in both the taxonomy of the fungus and the etiology of the disease.

SUMMARY

Sporangium formation by *P. parasitica* var. *nicotianae* was induced by incubating mycelium in a soil extract. The sporulation-inducing factor was found to be present in different soils and remained after ignition of these soils in a muffle furnace, hence is not organic. The optimum temperature for sporulation in a

soil extract was found to be 20°C to 25°C. Sporulation occurred at all pH values over the range of pH 5.4 to pH 11.0 with no clear optimum in this range. Light was without influence on sporulation.

A tenfold concentration of tap water induced abundant sporulation, but a hundredfold concentration was inhibitory to sporulation.

Materials effective in inducing sporulation could be eluted from charcoal by water or by dilute HCl. No sporulation occurred in dilute NaOH which had been used to elute charcoal. Distilled water eluates from charcoal were treated with anion- and cation-exchange resins with the result that the cation-exchange resin treatment did not affect the sporulation-inducing capacity of the eluate, but the anion-exchange resin treatment rendered the eluate inhibitory to sporulation.

A defined salt solution of the following composition which induced abundant sporulation was prepared: KCl, 50 mg.; KH_2PO_4, 100 mg.; $MgSO_4 \cdot 7H_2O$, 50 mg.; K_2CO_3, 300 mg.; and distilled water, 1000 ml. Apparently the induction of sporulation is due to the proper balance and concentration of inorganic ions in the medium, and the anion complex is of more importance in this respect than the cation complex. The presence of carbonates in the environment is indicated to be the determiner in inducing the sporulation response. The effect of pH could not be separated from the effect of components in the medium in inducing sporulation, and both probably interact to produce conditions suitable for the change from vegetative growth to sporangium formation.

It is suggested that the black shank fungus may be primarily a soil organism and only secondarily a pathogen. Variations could arise through crossing with other strains and with other species. Such variation in the fungus might help to explain breakdown in resistance of plants bred for resistance when considered in the light of other factors which might contribute to breakdown of resistance.

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TOXICITY AS A FACTOR IN TOBACCO BLACK SHANK[1]

BY FREDERICK T. WOLF[2] AND FREDERICK A. WOLF[3]

In an earlier paper (Wolf, 1933), evidence was presented indicating that the rapid wilting, yellowing and dying of leaves of tobacco plants having the black shank disease, caused by *Phytophthora parasitica* Dastur var. *nicotianae* Tucker (*P. nicotianae* Breda de Haan), was due to a toxin which the fungus metabolized. A reinvestigation of these findings appears to be desirable for several reasons. Chief among these is the fact that black shank has become widespread and has assumed major economic importance throughout the flue-cured tobacco areas of Georgia, South Carolina, North Carolina and Virginia, and has appeared also in burley tobacco districts of Kentucky and Tennessee. In addition, reports have been made to the writers that others have been unable to confirm these earlier findings. Powers (1952) performed some ingenious experiments involving (a) the recovery of wilted plants after making water accessible to them, and (b) the inability of diseased plants to conduct a dilute aqueous solution of eosine beyond the point of the lesion. This led him to conclude that "wilting is due to local obstruction of water movement through the vascular elements rather than to systemic effects of toxic metabolic products of the pathogen." He was careful to point out, however, that his findings do "not preclude the possibility that toxic substances are produced and are in some way responsible for this obstruction."

The present report embodies the results of recent studies of toxin production, as related to black shank. It was previously reported (Wolf, 1933) that the pathogen grows satisfactorily in potato broth, or in half-strength Richards' solution, and that tobacco plants wilted rapidly and succumbed when placed in either of these media following growth of the fungus, but failed to wilt in either uninoculated medium. It was concluded, therefore, that the culture filtrates contained a toxic product elaborated by the organism. It was also shown that a toxin may be demonstrated to be present in diseased plant tissues by grinding and extraction with water. This constitutes one of a very few cases of fungus disease of plants in which a vivotoxin (in the terminology of Dimond and Waggoner, 1953) has been demonstrated to occur within the tissues of an infected plant (Clauson-Kaas, Plattner and Gäumann, 1944). The precipitation of a toxic substance from an aqueous extract of diseased plants by magnesium sulphate provided the basis for suggesting that the toxin is a protein. Further experiments with culture filtrates indicated that the toxin was thermostable and non-volatile, and that the symptoms could not be accounted for on the basis of the changes of pH which occur in culture during growth of the pathogen.

MATERIALS AND METHODS

Eight isolates of *P. parasitica* var. *nicotianae* were used in these studies. Two were isolated from plants from the Tobacco Experiment Station, Oxford, N. C.

[1] We extend thanks to Dr. Alfred Ammann for his aid in this investigation.

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and one from each of the following places, Clay Station, Granville Co., N. C.; the greenhouse; Harrison and Christian Counties, Kentucky; Chatham, Virginia; and Hertford County, N. C. Stock cultures were maintained on slants of potato dextrose agar at room temperature, and were transferred at appropriate intervals.

A number of different media were used in tests of toxin production, as follows: (1) Potato dextrose broth prepared by boiling 100 g. of potatoes in 500 ml. of water for 20 minutes, removing the potatoes, and restoring to volume. A solution of twenty g. of dextrose in 500 ml. of water was then added. (2) Half-strength Richards' solution containing dextrose, 17 g.; KNO_3, 5 g.; KH_2PO_4, 2.5 g.; $MgSO_4 \cdot 7H_2O$, 1.25 g.; and a trace of $FeCl_3$, per liter. Additional nonsynthetic media included (3) oatmeal broth, containing 33 g. rolled oats per liter; (4) soybean broth, containing 30 g. soybeans per liter; (5) tobacco plant sap, prepared by chopping and grinding the non-woody parts of entire plants, filtering the juice and diluting with 4 volumes of distilled water; (6) starch glutamic acid medium based upon the findings of the nutritional studies of the black shank organism made by Wills (1952), which indicated that starch is an excellent carbon and energy source, and glutamic acid a fairly good nitrogen source for this fungus. This medium contained soluble starch, 10 g.; l-glutamic acid, 5 g.; KH_2PO_4, 1 g.; KCl, 0.5 g.; $MgSO_4 \cdot 7H_2O$, 0.5 g.; $FeCl_3$, trace; thiamine·HCl, 5 mg.; 0.2 M $Na_2HPO_4 \cdot 7H_2O$, 252.6 ml.; and 0.1 M citric acid, 147.4 ml., per liter. Other amino acids were substituted for glutamic acid in certain media.

Cultures were grown in 250-ml. Erlenmeyer flasks containing 50 ml. of medium, sterilized by autoclaving at 15 lbs. pressure for 20 minutes, and cultures were incubated at room temperature for varying periods of time.

Toxicity tests were performed with culture filtrates, from which the mycelia had been removed by forced filtration through filter paper in a Buchner funnel. The plant material for assay consisted of young detached leaves of greenhouse-grown tobacco plants, variety Hicks, and young leaves or branches of tomato, variety Marglobe. Toxicity tests were performed under uncontrolled conditions of temperature, humidity and light in the laboratory. Generally, the detached leaves were inserted in the culture filtrates in the late afternoon and the results were recorded the following morning. If necessary, the tests were prolonged for an additional day. Controls, using distilled water and uninoculated medium, were routinely included in all experiments.

RESULTS

TOXIN PRODUCTION IN VARIOUS MEDIA.—All strains of the pathogen failed to grow appreciably in half-strength Richards' solution, a finding at variance with the previous report (Wolf, 1933). To explain this discrepancy, it may be recalled that Robbins (1938) found that eleven species of *Phytophthora*, including *P. parasitica*, all require thiamine. It would seem reasonable to assume, therefore, that the dextrose used in our studies of twenty years ago may have been contaminated with thiamine. But findings by Wills (1952) indicated that inorganic nitrogen, either as nitrate or ammonium, is a very poor nitrogen source. Richards' solution supplemented with thiamine was found inadequate; hence experiments with this medium were discontinued.

The two strains from Oxford, N. C. were grown in oatmeal broth, and the filtrates were tested, after incubation for one, two, six, and nine and one-half weeks, for their capacity to induce wilting of tomato leaves. Likewise, two strains (Oxford, N. C. and Christian County, Ky.) were grown in soybean broth and assayed after one, two, four and seven weeks for capacity to wilt tomato leaves. With filtrates from cultures grown in oatmeal broth the findings were consistently negative. Soybean broth culture filtrates induced slight wilting, after one week for one strain, and after one and two weeks for the other strain, but wilting was never noted in filtrates over two weeks old. Although growth in these media was luxuriant, toxin production was negligible or was of doubtful occurrence.

Six strains of the fungus were grown in diluted tobacco plant sap. After incubation for two, four and six weeks, culture filtrates were tested for their capacity to wilt tomato leaves. The filtrates were used in dilutions of 1:1 and 1:10. The results with 1:1 dilutions are presented in Table I.

Little difference in toxin production was noted among the six strains tested in the tobacco sap. This medium tends to become rather highly alkaline, pH values being 8.0–8.1 after four weeks of incubation, and 8.1–8.4 after six weeks. It appears to provide for early development of toxin, since wilting of tomato

TABLE I

The degree of wilting induced in tomato leaves by culture filtrates of six strains of P. parasitica *var.* nicotianae *grown in diluted tobacco sap*

Strain	Age, Weeks	pH	Degree of wilting		
			Water control	Uninoculated medium	Culture filtrate
Tobacco Expt. Sta.	2	6.9	—	—	+++
	4	8.0	—	+	++++
	6	8.1	—	—	++
Oxford	2	7.7	—	—	+++
	4	8.1	—	+	+++
	6	8.2	—	—	++
Harrison Co., Ky.	2	7.5	—	—	++++
	4	8.0	—	+	+++
	6	8.4	—	—	++
Christian Co., Ky.	2	5.3	—	—	+++
	4	8.1	—	+	++
	6	8.4	—	—	++
Chatham, Va.	2	5.2	—	—	+++
	4	8.1	—	+	+++
	6	8.3	—	—	+++
Hertford Co., N. C.	2	5.3	—	—	+++
	4	8.0	—	+	+++
	6	8.3	—	—	++

TABLE II

The degree of wilting induced in tomato leaves by culture filtrates of three strains of P. parasitica *var.* nicotianae, *and various dilutions thereof, grown in starch-glutamic acid medium*

Strain	Age, Weeks	Dilution	pH	Degree of wilting		
				Water control	Uninoculated medium	Culture filtrate
Clay Station	2	1:1	4.9	—	—	—
	4	1:1	5.6	—	—	++++
		1:10			—	++
	7	1:1	6.7	—	—	++++
		1:10			—	+++
Greenhouse	2	1:1	4.9	—	—	—
	4	1:1	5.8	—	—	+++
		1:10			—	+
	7	1:1	6.6	—	—	++++
		1:10			—	++++
Oxford	4	1:1	4.8	—	—	++
		1:10			—	++
	6½	1:1	5.5	—	—	++++
		1:10			—	+++
		1:20			—	+
		1:50			—	+

leaves was induced in every instance in filtrate from two-week-old cultures. Culture filtrates in 1:10 dilution, however, failed to wilt tomato leaves, irrespective of the strain of fungus used, or of the length of the period of incubation. It is apparent, therefore, that the toxin was formed in small quantity only.

Results of similar experiments with three strains of the fungus grown in starch-glutamic acid medium are summarized in Table II. The pH changes are less than those in the tobacco sap medium because of the inclusion of a buffer. Toxin production does not proceed so rapidly in starch-glutamic acid medium as in tobacco sap, for wilting did not occur when two-week-old culture filtrates were assayed. More toxin is eventually formed, however, than in the tobacco sap medium. With the Oxford strain, after six and one-half weeks, it was found that filtrates would induce wilting in dilutions of 1:20 and 1:50. It is apparent, therefore, that the starch-glutamic acid medium is more nearly adequate for toxin production by the black shank fungus than is plant sap.

It appeared desirable to consider toxin production in other synthetic media, and, accordingly, glucose was substituted for starch in the starch-glutamic acid medium. Similarly, a number of amino acids were substituted for glutamic acid, each in an amount providing the same quantity of nitrogen, namely 480 mg. nitrogen per liter, as the glutamic acid in the original medium. No efforts were made to distinguish between *l-* and *dl-* amino acids. The results of these experiments are included in Table III.

Comparison of findings with the glucose-glutamic acid medium (Table III)

TABLE III

The degree of wilting induced in tomato leaves by culture filtrates of two strains of P. parasitica *var.* nicotianae *grown in media containing starch or glucose as the carbon source and various amino acids as the source of nitrogen*

Strain	Medium	Age, weeks	Dilution	pH	Degree of wilting		
					Water control	Uninoc. medium	Culture filtrate
Oxford	Glucose-Glutamic acid	4	1:1	4.6	—	+	++++
			1:10			—	++++
		6½	1:1	5.0	—	—	++++
			1:10			—	++++
			1:20			—	++
			1:50			—	—
Clay Station	Starch-*dl*-Alanine	4	1:1	6.0	—	—	++++
		6	1:1	6.5	—	—	++++
Clay Station	Starch-*dl*-Aspartic acid	4	1:1	5.5	—	—	—
		6	1:1	5.8	—	—	+++
Clay Station	Starch-*l*-Leucine	4	1:1	5.5	—	—	—
		6	1:1	5.5	—	—	++++
Clay Station	Starch-*dl*-Valine	4	1:1	5.6	—	—	—
		6	1:1	6.0	—	—	+++
Clay Station	Starch-*l*-Tyrosine	4	1:1	5.9	—	—	—
		6	1:1	6.0	—	—	++++
Clay Station	Starch-*l*-Proline	4	1:1	6.1	—	—	++++
		6	1:1	6.6	—	—	++++
Clay Station	Starch-*l*-Arginine-HCl	4	1:1	5.5	—	—	++++
		6	1:1	6.6	—	—	++++
Clay Station	Starch-*l*-Histidine·HCl	4	1:1	5.5	—	—	++++
		6	1:1	6.5	—	—	++++
Clay Station	Starch-*dl*-Ornithine·HCl	4	1:1	5.9	—	—	—
		6	1:1	5.7	—	—	—

and the starch-glutamic acid medium (Table II) may be made in the case of the Oxford strain of the fungus, using comparable incubation periods (four, and six and one-half weeks). Such comparison shows little difference, although the intensity of the symptoms evoked with four week old culture filtrates of glucose-glutamic acid is greater than that in filtrates of starch-glutamic acid medium of equal age, suggesting earlier formation of a higher toxin titer in the medium containing glucose. With six and one-half weeks old culture filtrates, a 1:50 dilution of glucose-glutamic acid culture filtrate failed to cause wilting whereas a comparable dilution of the starch-containing medium, as previously stated, induced wilting.

The experiments in which various amino acids were used as replacements for glutamic acid in the starch-glutamic acid medium (Table III) employed the Clay Station strain, incubated for four and six weeks, and comparable data for glutamic acid may be found in Table II. Unfortunately, no dilutions greater than 1:1 were tested. Toxin production is evident within four weeks in media containing alanine, proline, arginine, or histidine. Tests were negative at four weeks, but positive after six weeks in the case of aspartic acid, leucine, valine, and tyrosine. Toxin was not detected after six weeks with ornithine as the nitrogen source. Although measurements of amount of mycelial growth were not made it appears that amount of growth and toxin production are correlated. Greatest growth of the fungus occurred in those media in which toxin was found after six, but not after four weeks.

TOXIN IN DISEASED PLANTS.—In our previous paper (Wolf, 1933), as has been stated, toxin was demonstrated to be present in black shank-infected plants. Because of the interest in vivotoxins (Dimond and Waggoner, 1953) which have been demonstrated in relatively few instances in diseased plants, further experiments were devised to provide additional evidence of the possible presence of the toxin in black shank tissues.

Accordingly, mature infected plants were collected in the field, and blackened tissues of the stem cortex and pith were removed as small pieces. This material was then passed through a food chopper. The resulting juice, and that from the tissues macerated by squeezing through several thicknesses of cheesecloth, were combined. This product was then filtered through filter paper in a Büchner funnel. Tissues of healthy plants were prepared in the same way, to yield healthy sap for use as a control.

Small leaves of greenhouse-grown tobacco and tomato plants were used for the assays. It was found that sap from healthy tobacco plants would induce a wilting of tobacco or tomato leaves, as would also the sap from black-shank-infected tissues. With diseased sap, wilting was accompanied by discoloration of the vascular tissues. The differences in appearance between the two sets of leaves were not sufficiently pronounced to afford unequivocal proof of the presence of toxin. Attempts to demonstrate toxin in the leaves of diseased plants by the same technique also gave results which were not decisive.

In an experiment, to be described later, it was found that the toxin present in culture filtrates could be removed from the filtrates by adsorption on charcoal.

Accordingly, samples of sap from healthy and diseased Hicks tobacco plants were prepared as previously described, using the cortex and pith of the basal portions of the stem in each instance. A portion of the sap from diseased plants was treated with charcoal, the charcoal then being removed by filtration. Into the samples of healthy sap, diseased sap, and charcoal-treated diseased sap, were inserted leaves of tobacco (Hicks variety), tomato, *Nicotiana longiflora* Cav., *N. plumbaginifolia* Wild., and *N. rustica L.* Wilting of tobacco, tomato, and *N. longiflora* leaves occurred within one hour in the case of all three treatments, but there was no evidence of toxicity in leaves of *N. plumbaginifolia* and *N. rustica*. At the end of one hour, leaves were removed from each sample of tobacco sap and placed in distilled water. It was noted twenty-four hours later that those

leaves which had wilted in healthy sap or in charcoal-treated diseased sap had fully recovered, whereas wilting had become severe in the leaves of tobacco and tomato placed in the diseased sap not treated with charcoal. Recovery of *N. longiflora* leaves was complete, regardless of the source of sap or its treatment. Leaves of *N. rustica*, which gave evidence of wilting after a one-hour exposure, remained turgid, but leaves which had been inserted in the untreated diseased sap for one hour became flaccid. A reasonable interpretation of these findings would appear to be as follows: Since charcoal adsorbs toxin from culture filtrates of the fungus, *in vitro*, it might be expected to act similarly upon toxin formed *in vivo*. The results obtained with tobacco and tomato fully support this assumption. The initial wilting may be attributed to a constituent present in healthy (and also in diseased) plants, which is not adsorbed by charcoal. Recovery from wilting induced by this constituent occurs when the test leaves are transferred to distilled water. Such wilting may be regarded as physiologic wilting. The non-recovery of leaves wilted by the sap from diseased plants, not charcoal-treated, would appear to be a clear demonstration of the presence of a vivotoxin, and such wilting may be regarded as pathologic wilting. That toxin is produced is supported also by the results with leaves of *N. rustica*, which were not wilted after a one-hour exposure to diseased sap, but which became wilted and remained wilted after being placed in distilled water. Apparently such leaves absorbed sufficient toxin in a one-hour exposure to cause subsequent wilting.

Additional evidence for a vivotoxin was provided by an experiment of a different type. Healthy and black-shank-infected tobacco plants were cut off near the base of the stems. A piece of glass tubing was attached in a vertical position to each stump, the connection between the stem and the glass tubing being made with rubber tubing. The upper ends of the glass tubes were attached to a manifold which, in turn, was connected to a water aspirator. A suction of $\frac{1}{5}$ atmosphere, as measured by a manometer, was applied to the system. The liquid withdrawn, by this appliance, from the cut upper ends of the stems, was collected in the glass tubes. The fluid extracted from diseased plants was much less in volume than from healthy plants, necessitating the pooling of samples from several diseased plants in order to obtain a sufficient volume of material for testing.

When tobacco leaves were inserted in vials containing these fluids, it was found that wilting occurred in fluid from diseased plants, but not in that from healthy plants. This technique, somewhat similar to the centrifugation method used by Gottlieb (1943, 1944) in his studies of tomato wilt caused by *Fusarium oxysporum* f. *lycopersici*, eliminates the consideration of the cellular constituent of healthy plants which caused wilting in our earlier experiments, and thus affords a more direct proof of the existence of a toxin within the tissues of black-shank-diseased plants.

PROPERTIES OF THE TOXIN PRODUCED IN CULTURE.—As previously reported (Wolf, 1933), the pathogen grows satisfactorily in potato broth, in which it produces materials which are precipited by magnesium sulphate, suggesting that they are protein and that the toxin itself may be a protein. Additional previous

findings disclosed that the toxin was thermostable and non-volatile. Thermostability of the toxin appears unlikely if the toxin is protein in nature. Accordingly, the properties of the toxin were reinvestigated in a series of experiments, using the strain obtained from the Tobacco Experiment Station, grown in potato dextrose broth. In preliminary tests, on this medium, it was found that four weeks' growth was ordinarily required before the culture filtrate of this strain would bring about wilting of detached leaves of tobacco or tomato. Again all toxicity tests involved distilled water controls, and uninoculated potato broth controls.

Wilting was regularly induced by filtrates which had been boiled for fifteen minutes. When, however, the filtrate was evaporated to dryness, and then made up to the original volume with distilled water, no wilting resulted. It is apparent, therefore, that the toxin present in filtrates has a moderate degree of thermostability, but can be destroyed by prolonged boiling.

In attempts to ascertain whether the toxin was volatile, 75 ml. of filtrate were subjected to distillation, and three successive 10 ml. fractions of the distillate were collected. Each fraction was assayed and failed to bring about wilting, but a sample of the liquid remaining in the distilling flask was found to have retained its toxic character, although the symptoms produced were not so marked as when the unheated filtrate was used. It may be concluded, therefore, that the toxin is non-volatile.

Portions of culture filtrate were enclosed in "Visking" cellophane sausage casing and dialyzed overnight against running water. Then the contents of the casing, and also a portion of undialyzed culture filtrate, were tested for capacity to produce wilting in tomato leaves. The undialyzed filtrate brought about wilting, whereas dialyzed filtrate did not. The toxic material is therefore dialyzable, suggesting that it is constituted of a substance of small molecular size.

To culture filtrate, solid finely-ground $MgSO_4 \cdot 7H_2O$ was slowly added until a flocculent precipitate occurred. The precipitate was removed by filtration, redissolved in distilled water of sufficient amount to restore the original volume, and samples of this material were assayed for wilting activity, with negative results. Because of its high salt content, the filtrate remaining after separation of the flocculent precipitate was not tested.

In a similar experiment, 95 per cent ethyl alcohol was added to filtrate, in a quantity calculated to bring about a final concentration of 70% alcohol. The flocculent precipitate which resulted was collected by filtration, redissolved in distilled water, and assayed for wilting activity. This precipitate too was devoid of wilting activity. These experiments, considered together with the results obtained upon dialysis of the culture filtrate, indicate that the toxin is not a protein.

Also, culture filtrates were extracted with small quantities of ethyl ether in a separatory funnel, and the ether solutions were separated from the aqueous layer of filtrate and pooled. The filtrate, after extraction with ether, was found to induce severe wilting of test plants. The ether solution was evaporated to dryness, and the residue was taken up in a small volume of water and tested for wilting capacity, with negative results. Since the boiling point of ether is so

low as to preclude the possibility of destruction of toxin by heat during evaporation of the ether, it may be concluded that the toxin is insoluble in ether.

Filtrate was tested for the presence of nitrite with sulfanilic acid and alpha-naphthylamine, with negative results.

To a sample of culture filtrate was added an equal volume of 0.2 N barium hydroxide solution and a precipitate formed which presumably contained phosphates, sulphates, and organic acid salts of barium. The precipitate was removed, and the filtrate, after neutralization with hydrochloric acid, induced wilting. This result suggests that the toxin is probably not an organic acid.

A 200-ml. sample of the filtrate was concentrated by vacuum distillation at a temperature of 50–55°C. to a volume of 5 ml. At this point it was noted that a crystalline material was being precipitated from the concentrated solution. The crystals were collected by centrifugation and decantation, and were dissolved in a small volume of water. Upon testing, it was found that the solution of the crystalline material induced wilting, but even more severe wilting and necrosis were noted in plants in the concentrated culture filtrate from which the crystals had been removed. Thereupon another 200-ml. sample of filtrate, as well as an equal quantity of uninoculated potato dextrose broth, were evaporated almost to dryness. Crystals which were subsequently identified as calcium oxalate, appeared both in the concentrated filtrate and in the concentrated uninoculated medium. Obviously the source of this material was the potatoes, and the finding of calcium oxalate crystals in the uninoculated medium indicated that it was not a metabolic product of the fungus. The fact that toxicity of culture filtrates is largely destroyed by boiling to dryness would likewise support the idea that the toxin is not an oxalate.

Filtrates were shaken with charcoal, and after a few minutes the charcoal was removed by filtration, whereupon portions of the original filtrate and the charcoal-treated filtrate were assayed. In many repetitions of this experiment, it was found that charcoal treatment results in the complete loss of wilting capacity. The toxin is therefore adsorbed on the charcoal.

Attempts were then made to elute adsorbed toxin from the charcoal. Charcoal with adsorbed toxin was shaken with a one per cent solution of NH_4OH. The charcoal was then removed by filtration, and the solution was neutralized, and assayed with negative results. Similarly, one per cent acetic acid was tried, and after removal of the charcoal and neutralization, it was found that this solution induced injury in test plants, not identical, however, with the symptoms induced by untreated filtrates. Further study indicated that these findings could be duplicated by a neutralized one per cent solution of acetic acid. Experiments in which one per cent HCl was substituted for acetic acid gave inconsistent and inconclusive results.

Elution of the toxin from charcoal by means of various organic solvents was then tried. Acetone, ether, or 95 per cent ethyl alcohol were shaken with charcoal on which toxin had been adsorbed. The charcoal was removed by filtration, the organic solvents were taken to dryness, and the residues were dissolved in water and tested, with negative results. In additional experiments, attempts

were made to elute the toxin with benzene, petroleum ether, or 30 per cent aqueous pyridine, and the solvents were removed by vacuum distillation, in order to minimize possible toxin destruction by heat. Results were consistently negative, and all attempts to elute the toxin from charcoal have failed.

As previously noted, all of these experiments to determine the properties of the toxin employed filtrates from cultures grown in potato dextrose broth. Experiments were also made with filtrates of cultures grown in starch-glutamic acid medium. The results concerning thermostability, non-volatility, ether insolubility, and charcoal adsorption of the toxin confirmed the findings with potato dextrose broth, and hence are not presented in detail. So, despite considerable effort, the chemical nature of the toxin of *P. parasitica* var. *nicotianae* remains quite unknown.

DISCUSSION

No evidence has been secured to indicate that *P. parasitica* var. *nicotianae* produces toxin in oatmeal broth or soybean broth although growth was excellent in these media. Evidence favoring the production of toxin *in vitro* was obtained, however, when the fungus was grown in diluted tobacco plant sap, in potato dextrose broth, or in a starch-glutamic acid synthetic medium. Modifications of the latter medium by substitution of glucose for starch, and by substitution of any one of nine amino acids for glutamic acid, indicate that toxin production occurs on a wide variety of synthetic media. The only instance in synthetic media in which no toxin was found was in the medium containing ornithine as the nitrogen source, in which growth was so poor as to preclude the likelihood of toxin biosynthesis. The question of possible specific toxin precursors thus arises. Data obtained on the synthetic media tend to support the idea that no specific precursor exists and the failure to find toxin in oatmeal broth or soybean broth, irrespective of duration of incubation, suggests that if a precursor exists these media lack it.

The demonstration that wilting may be induced using a dilution of culture filtrate as great as 1:50 suggests that any explanation of wilting as a result of osmotic activity alone of the culture medium can have little factual basis.

The experiments involving studies with black shank-infected tissues tend to strengthen the previous finding (Wolf, 1933) that a toxin is present in diseased plants. According to Clauson-Kaas, Plattner and Gäumann (1944) our previous report was the only instance known at that time in which a vivotoxin had been demonstrated. Dimond and Waggoner (1953) have recently reviewed the literature concerned with vivotoxins. The studies of Gottlieb (1943, 1944) with *Fusarium oxysporum* f. *lycopersici* provide evidence for the presence of a vivotoxin in plants infected with tomato wilt. The voluminous studies of Gäumann and others on this disease, in the course of which lycomarasmin, fusarinic acid and a polysaccharide have been identified as factors involved in the wilting complex, have failed to produce evidence favoring the metabolism of any of these *in vivo*. That a toxin is produced *in vivo* by the tomato fungus was shown recently by Davis (1954). He grafted potato, tobacco, nightshade, pepper, and

Physalis on tomato stocks, inoculated the stocks with the tomato Fusarium, and noted that the scions wilted in many cases before wilt symptoms were apparent in the stocks. The pathogen did not invade these non-tomato scions.

The work of Litzenberger (1949) with the Victoria blight of oats, caused by *Helminthosporium victoriae* Meehan & Murphy, indicates that a vivotoxin is produced in this disease, but the chemical nature of the toxin is not yet known. Evidence of the existence of vivotoxins in other diseases remains inconclusive.

Further study is required to determine the chemical nature of the toxin or toxins herein investigated, but the results provide a starting point for future biochemical investigation.

SUMMARY

This study is concerned with toxin production by *Phytophthora parasitica* var. *nicotianae* in culture, with the demonstration of toxin within tissues of infected tobacco plants, and with the properties of the toxin or toxins. The wilting of detached leaves of tomato and tobacco was used as a method of assay of toxin. No evidence of toxin was found when the black shank organism was grown in oatmeal broth or soybean broth. Filtrates of cultures grown in diluted sap of tobacco for two weeks or longer, or filtrates of cultures grown in potato-dextrose broth or in a starch-glutamic acid synthetic medium for four weeks or longer regularly induced wilting. Wilting occurred in low dilutions, but also in dilutions of 1:5 or 1:10. Wilting was induced by a 1:50 dilution of the filtrate from a culture grown on the starch-glutamic acid medium for six and one-half weeks. The substitution of glucose for starch, or other amino acids for glutamic acid had little influence upon toxin production, so long as the organism grew at least moderately well in the medium.

Attempts to demonstrate the toxin in diseased plants by grinding and extracting in water were not wholly satisfactory because wilting was also to be found in controls prepared from healthy plants. Toxin was demonstrated, however, in black shank-infected plants in an indirect manner by a technique which involved adsorption of toxin on charcoal, and transfer of the test leaves to distilled water following short periods of contact with the products of ground diseased tissues. Toxin was also demonstrated in the fluids removed by suction from the stems of diseased plants.

The toxin produced in culture filtrates of the pathogen is moderately heat stable, non-volatile, and dialyzable. It is not a protein, nitrite or oxalate, is probably not an organic acid, and is adsorbed from culture filtrates by charcoal. Efforts to elute it from the charcoal have been unsuccessful.

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THE TRANSPIRATION OF BLACK SHANK-INFECTED TOBACCO

BY ROBERT J. SCHRAMM, JR.[1] AND FREDERICK T. WOLF[2]

Relatively few studies have been made of transpiration of diseased plants. One of the early studies of this kind is that of Reed and Cooley (1912). They determined that the transpiration of York Imperial apple leaves infected by the rust *Gymnosporangium juniperi-virginianae* Schw. is 78.3%, and that of Ben Davis apple leaves 72.4%, as compared with that of normal apple leaves. The more recent studies of water losses by diseased plants involve soil-inhabiting, vascular-tissue-invading bacterial and fungus pathogens which produce the syndrome called wilt.

As a result of studies of certain wilt diseases of field and garden crops, especially tomato wilt, induced by *Fusarium oxysporum* f. *lycopersici* Snyder and Hansen, two theories have been proposed and evaluated to account for the proximate cause of wilting. These theories are that (1) wilting is a consequence of occlusion of the vessels, arising from the abundance of mycelium within the vessels and/or from tyloses or gums arising from interaction of the pathogen and suscept (Ludwig, 1952; Scheffer and Walker, 1953; Dimond and Waggoner, 1953) and that (2) the pathogen metabolizes a toxin or toxins. The toxin theory, as elaborated by Gäumann (1951) and associates, ascribes the cause of wilting of tomato to a polypeptide, lycomarasmin, and to fusarinic acid. That these products are formed by the pathogen *in vitro* is well founded but evidence of their production *in vivo* is less well established. The wilting of non-tomato scions grown on tomato stock (Davis, 1954), however, offers very convincing evidence of production of toxin, *in vivo*, by the tomato pathogen.

Wilt is among the symptoms in the complex of symptoms of tobacco black shank induced by *Phytophthora parasitica* var. *nicotianae* Tucker. The present

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study of wilting involves a comparison by transpirational losses of black shank-affected tobacco plants and healthy ones. Major emphasis was centered on determining the extent to which transpiration is modified and on efforts to learn something of the modificatory mechanisms.

MATERIALS AND METHODS

The tobacco variety Gold Dollar was selected for use because it is known to lack appreciable resistance to attack by *P. parasitica* var. *nicotianae*. Plants grown in seedbeds and of a size suitable for transplantation into the field were transplanted into 6-inch unglazed pots and kept in the greenhouse. When they had grown to be about 12 inches tall, groups of 6 to 20 plants were selected for uniformity, one group being used for controls and those of the other for inoculation.

The inoculum consisted mainly of cultures grown on moistened sterilized oat grains. Another medium which supported good growth of the pathogen for use as inoculum was potato broth.

It is known from observation that the severity of black shank is conditioned by several factors including temperature, rainfall, and injury resulting from mechanical breakage of the roots, from nematodes, from fertilizers, and from other soil-borne pathogens. The present studies were made difficult because it was not found possible to induce black shank at will in all inoculated plants, regardless of efforts to employ these modifying factors.

Some of the experiments were conducted outdoors and others in the greenhouse or laboratory. In some cases no effort was made to control temperature, humidity, or even water loss from the surfaces of the soil and of the pots. In certain experiments various devices to prevent water loss except through transpiration were employed, as mentioned subsequently. A continuous record of temperature and relative humidity was obtained in certain experiments, by use of a hygrothermograph.

"Leaf area", as used herein, applies to measurements made by means of an instrument employing a photoelectric cell. Objects placed in the path of the light beam interfered with the passage of light, the amount of interference being a function of the area of the object. Using zero to represent no interference it was possible to calibrate and to graph the amounts of interference, designating the results as leaf area.

Dry weights of leaves were obtained by desiccation to constant weight in an oven having a temperature of 115° Fahr.

RESULTS

It was realized at the outset that measurements involving water loss of greenhouse-grown plants might have little value in an interpretation of transpirational losses by field-grown plants. And it was realized also that it would be impossible to establish fixed values to represent the transpiration of greenhouse-grown diseased plants and healthy plants under all conditions. Among the reasons are that the rate of water loss would be related to the stage of the

disease, and that there are difficulties attendant on evaluating separately the modificatory effects of light, temperature, relative humidity, soil moisture supply, and aeration of the soil. A number of preliminary experiments were conducted in efforts to circumvent these difficulties. When, for example, 3 normal plants and 3 having black shank lesions near the soil level were weighed hourly and the results were compared it was found that the average water loss from diseased plants was 1.01 ml./dm² leaf area/hr. and that from healthy ones was 2.06 ml./dm² leaf area/hr. In this case no effort was made to measure the water lost from the soil surfaces and pot surfaces apart from that lost by transpiration. In another test, in order to eliminate water loss except by the plants themselves, the potted plants were placed in galvanized iron cans sealed with plastic covers. This experiment extended over a five-hour period, weighings being made each hour. As a result, the average transpiration rate from black shank-affected plants was 0.24 ml./dm² leaf area/hr. and for healthy plants, 1.88 ml./dm² leaf area/hr.

In a third series, involving 12 plants, the weighings were made less frequently but the total period extended over 43 consecutive hours. Again, water loss, except by transpiration, was guarded against by placing the pots in metal cans and covering the exposed surfaces with pieces of plastic. In this instance the average transpirational loss amounted to 0.053 ml./dm² leaf area/hr. from diseased plants and to 0.293 ml./dm² leaf area/hr. from healthy plants.

The results of each of the foregoing preliminary experiments were alike only in indicating that water loss from healthy plants exceeds that of diseased plants. These water loss differences involved not only the two groups of plants as units but also each member within each group differed from each other in terms of loss from unit area of leaf surface. To account for the differences between groups and among the plants of each group of the three foregoing experiments, it was surmised that the stage of the disease might be a factor. Accordingly, efforts were directed toward following the course of water loss coincident with the progress of the disease. This experiment involved 20 plants. The fungus, grown on oat grains, was incorporated with the soil around 10 plants, and the other 10 plants served as controls. On the third day after inoculation, the potted plants were placed in metal cans provided with metal covers which were tightly sealed with wax. A hole in the cover, which was closed with a rubber stopper, provided an opening for periodic watering. These plants were kept for 2 weeks in a well lighted room having temperatures maintained within the range 82–95°Fahr. and relative humidities of 70 to 80%, conditions known to favor development of the disease. But under these conditions the plants grew poorly and only 3 of the 10 plants became infected. Apparently the lack of an adequate supply of oxygen to the roots interfered both with root development and with growth of the pathogen. All efforts were therefore abandoned to control temperature and relative humidity and also to seal the cans so tightly.

Accordingly, in succeeding experiments less closely-fitted oil cloth covers were used. They were kept in place during the daytime when periodic weighings were made and were removed each night. The average data for water losses by the control group on the 8th, 11th and 12th day after inoculation are recorded in Fig. 1. A daily summation for 5 consecutive days is shown in Fig. 2.

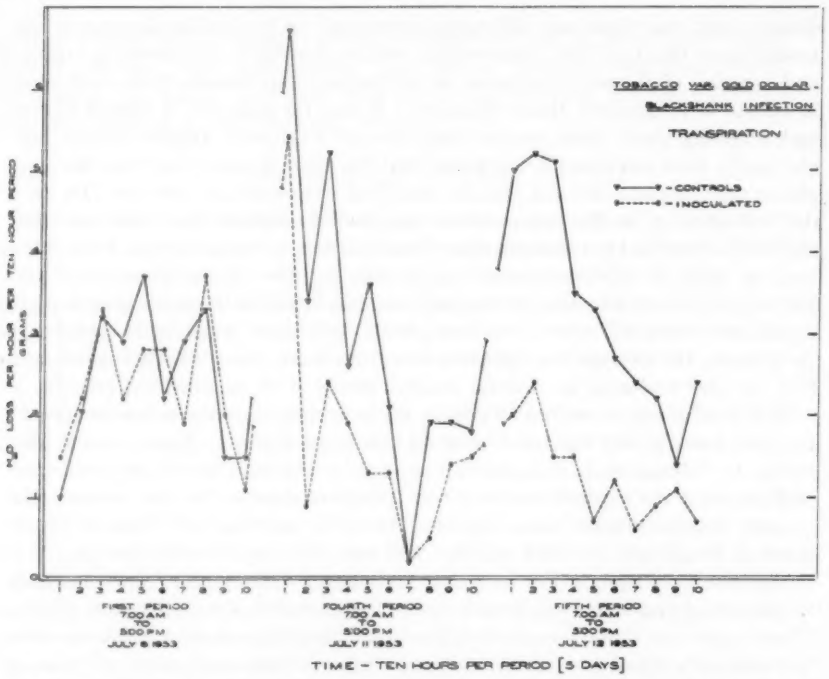

FIG. 1. Comparative water losses of healthy plants and black shank-affected ones for eight-hour periods on the 8th, 11th, and 12th days after inoculation.

The main features exhibited by these graphs are the greater water loss by healthy plants, as in all previous experiments, and additionally that the comparative differences in transpiration between normal plants and diseased ones increase as the disease progresses.

After it had been established that the transpiration of healthy plants exceeds that of black shank-affected ones, efforts were made to compare water absorption by roots of diseased and healthy plants since transpiration and absorption are known to be so intimately interdependent.

Accordingly the stems of healthy and diseased plants (Fig. 3) were severed near the soil level and the cut stumps were attached by means of rubber tubing to graduated glass tubes (manometers) connected in a series. A suction force of 0.2 atmospheres was provided to evacuate the air from the system and to pull water through the cut ends of the stems. Readings of the volume of liquid pulled through the roots were made at intervals over a period of 4 hours. In all cases considerable quantities of liquid were pulled through the stumps of healthy plants, but none or only a small amount was pulled through the stumps of diseased plants. Manifestly the vessels of the black shank-affected plants were always more or less occluded.

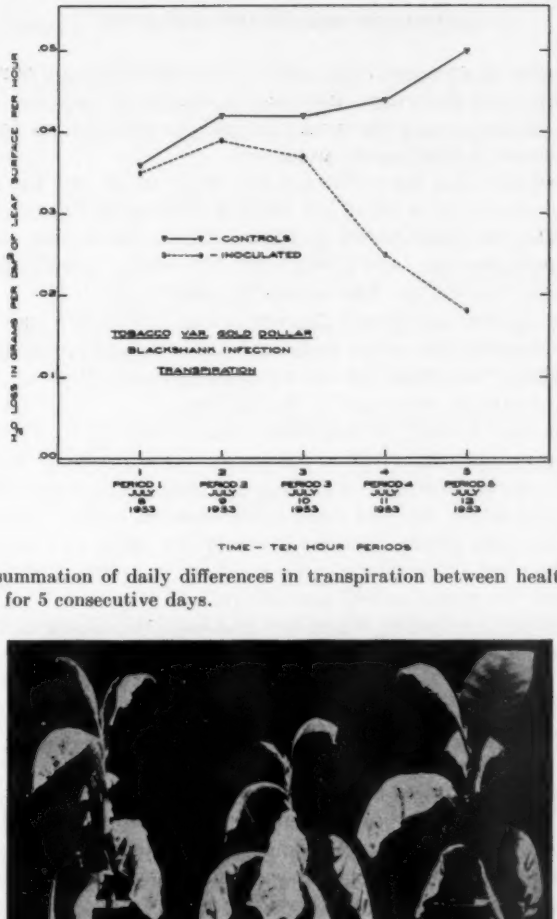

FIG. 2. A summation of daily differences in transpiration between healthy plants and diseased ones for 5 consecutive days.

FIG. 3. Comparison of black shank-affected tobacco (above) with healthy plants (below). The lower leaves of the diseased plants are yellowish and wilted, those immediately above are wilted only, and the bud leaves are turgid.

Whether degree of occlusion of the vessels is a correct measure of root absorption in accounting for the entire difference in amount of transpiration between diseased and healthy plants, however, is open to question, as indicated by several other series of experiments as follows.

In a series of one kind the roots of plants were cut off and the cut ends of the stems were submerged in one of four kinds of solutions in Erlenmeyer flasks—(a) distilled water, (b) uninoculated potato broth, (c) filtrate from potato broth in which the pathogen had been grown for 4 to 8 weeks, and (d) filtrate as in (c) but to which charcoal had been added to adsorb toxin.

In a series of another kind intact plants were employed which had been grown for several weeks with the roots suspended in Hoagland's nutrient solution. These intact plants were transferred to solutions similar to those in the previous series, their roots being submerged in the solutions.

The experiments involved in both series were conducted in the greenhouse. Records of water loss were made at intervals over a period of 8 consecutive hours. The results showed that in both series most rapid losses involved the plants in distilled water, the least rapid losses occurred in the filtrates in which *Phytopthora* had been grown, and the losses in the other two solutions were essentially alike and intermediate between those in water and those in the filtrate in which the pathogen had been grown.

These results are interpreted to indicate that toxic substances and concentrations of osmotically active materials were both operative in modifying transpiration.

DISCUSSION

Obviously any hypothesis that accounts for the wilting of the lowermost leaves of black shank-affected tobacco plants must at the same time account for the fact that the tip or "bud-leaves" remain turgid even when the lower leaves have become very flaccid. It is a well known fact that young leaves of tobacco are able to translocate, to a remarkable extent, water from older leaves. The existence of an osmotic gradient, therefore, may be invoked to account for turgidity of tip-leaves when the lower leaves are wilted. But this leaves unexplained the differential upward movement of water and toxin. There is evidence in hand to show that absorption of water is impeded in diseased roots, that the vessels at the base of the stem become occluded, and that toxic materials elaborated in fluid cultures interfere with uptake of water and with transpiration. Also the evidence shows impediment of water flow in the stems and that obstruction increases as infection progresses.

Manifestly, therefore, both the occlusion hypothesis and the toxin hypothesis are involved and are complementary in accounting for decreased transpiration of black shank-affected tobacco plants.

SUMMARY

Black shank-affected tobacco plants transpire at a slower rate than do healthy ones. As the disease progresses the transpirational losses decrease. Among the

interrelated factors involved in such impediment of water loss are progressive destruction of the roots, occlusion of the vessels, and elaboration of a toxin or toxins which are produced by the interaction of the pathogen and the suscept tissues.

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RESPIRATION OF TOBACCO BLACK SHANK TISSUES

BY FREDERICK T. WOLF[1] AND ROBERT J. SCHRAMM, JR.[2]

Conceivably a knowledge of the ability of a pathogenic fungus to modify respiration could be employed to understand better the pathological processes which the causal agency induces. Very few studies of the respiratory activity of diseased plant tissue have been made, however, presumably because of the technical difficulties involved.

Among investigations having relevancy are those of Nicolas (1920), Yarwood (1934), Dufrenoy and Dufrenoy (1934) and Allen and Goddard (1938), which deal with fungus diseases, and those of Glasstone (1942), Wynd (1943) and Löhr and Müller (1952), which deal with virus diseases. Nicolas (1920) reported a decrease in respiration of *Prasium majus* L. attacked by the powdery mildew, *Erysiphe lamprocarpa* Wallr. and of *Torilis nodosa* Kotschy attacked by *Erysiphe communis* (Wallr.) Fr. Yarwood (1934), using excised clover leaflets floated on sucrose solutions, found that *Erysiphe polygoni* (Wallr.) Lév. induced an increase in respiration of 41% and that *Uromyces fallens* Kern, a rust, induced an increase of 123% during the 9- to 11-day period after inoculation. Allen and Goddard (1938), by use of a Fenn respirometer, compared the respiratory activities of normal wheat leaf tissue, mildewed leaf tissue, and mildewed leaf tissue from which the pathogen had been removed by brushing carefully with a camel's hair brush. They found that the rate of respiration of mildewed tissues is always greater than that of normal tissues, the maximum difference being as great as

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650%. The larger proportion of such increase in gaseous metabolism occurred in the affected mesophyll tissues themselves.

Dufrenoy and Dufrenoy (1934), using a Warburg respirometer, found a higher respiratory intensity in tobacco mosaic than in normal tissues. The studies by Glasstone (1942) and Wynd (1943) confirmed these findings and made the additional observation that elapsed time after inoculation is a factor in conditioning the respiratory rate in tobacco mosaic. Glasstone devised a respirometer to use entire plants. Löhr and Müller (1952) noted that the rate of respiration of virus-infected sugar beets is higher than that of healthy ones.

Whether the total oxygen consumption by virus-affected plants is due to tissue respiration alone, or arises in part from the virus, remains uncertain because of lack of accord among virologists on whether viruses are living entities.

METHODS

A Warburg respirometer was employed in the present studies. After preliminary tests with small pieces of cortical tissue and thin disks of pith tissue, both from diseased plants and from healthy ones, it was decided, because all of the pith cells were quite alike, to employ only pith tissues. Also, since these preliminary tests showed a rather large plant-to-plant difference, it was decided to use both "healthy" and diseased pith taken from the same plant. Field grown plants, a Turkish variety and a flue-cured variety, Gold Dollar, provided the materials.

Readings with each sample were made at 10-minute intervals and were continued for a period of 2 hours. The tissues used in each experiment were carefully dried to constant weight after the series of readings had been made. If for any reason the rates of increase in O_2 consumption were erratic the data were discarded, only those which showed an essentially straight-line cumulative increase being retained. By calculation, the QO_2, was derived from these data (Warburg readings) and those on dry weight of tissues.

RESULTS

Representative data on respiratory rates of healthy pith tissues in comparison with black shank-affected tissues, as measured by the Warburg apparatus, are assembled in Table I.

TABLE I

Comparative respiration of healthy tobacco pith tissues and black shank-affected pith tissues

No. of trials	Average QO_2 of healthy tissue, mm^3/hr./mg. dry tissue	No. of trials	Average QO_2 of black shank affected tissue, mm^3/hr./mg. dry tissue	% change
3	1.64	2	6.48	+294
3	1.32	1	2.68	+103
3	1.46	2	2.07	+42
1	2.31	1	4.37	+89
2	2.41	1	3.71	+54

It is evident, first of all, that the respiratory rate of infected pith tissues is consistently greater than that of normal tissues, the percentage increases shown ranging from 42 to 294. These differences appear to be correlated mainly with the stage of the disease. Obviously it is not possible to follow sequentially these changes, but certain measurements, not tabulated herein, involving different plants in different stages of disease, indicate that the respiratory rate is maximal early in the disease and subsequently declines.

Also it may be pointed out that no method is available to evaluate the QO_2 of pith tissues apart from that of the pathogen. However, comparison of the respiratory rates of young and old cultures of the black shank fungus was made with the result that the QO_2 of young cultures was found to be 6 to 7 times larger than that of old cultures. A similar difference in rate of respiration between young and old cultures of *Penicillium notatum* has been reported by Wolf (1947). Moreover, no method for securing direct proof is presently available to determine whether the high respiratory rate of black shank pith tissues, early in the disease, is mainly due to the respiration of the pathogen.

DISCUSSION

The fact that black shank-affected pith tissues respire at a higher rate than healthy tissues accords with expectations. Proof is lacking, however, as to whether other invaded host tissues, such as cortex and leaf tissues, respond similarly. Since it has not been possible to separate the respiration of the tobacco tissues from that of the pathogen, nothing is known quantitatively about the respiratory rate of each of the two components.

It may be presumed that the stores of energy within invaded tissues are depleted through respiratory activity. If such is the case there might remain insufficient energy for "active absorption" of water by the roots of diseased plants, as is reflected by decrease in transpiration (Schramm and Wolf, 1954). There might also occur, as another consequence, an interference with reserves of carbohydrates and proteins in the leaves, as will be established in a subsequent report.

SUMMARY

Comparison of respiratory rates of black shank-affected tissues and healthy pith tissues discloses that the rates are appreciably higher for invaded tissues. It has not been possible to separate that increase in rate for which the pith cells may be responsible from that due to the invading pathogen. The rate of respiration appears to change during the course of the disease, being maximal early in the disease.

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THE CARBOHYDRATE AND NITROGEN METABOLISM OF BLACK SHANK-AFFECTED TOBACCO[1]

BY FREDERICK A. WOLF[2] AND FREDERICK T. WOLF[3]

INTRODUCTION

Plant pathological literature is replete with data showing reduction in yield and quality of crop plants resulting from ravages of diseases. Such reduction reflects the extent to which all of the metabolic activities of the suscept have been modified. Strangely, however, relatively little consideration has been given to determination of the extent to which individual physiologic processes, which together constitute the suscept's metabolic activity, are altered. The present report deals with efforts to learn whether the black shank fungus, *Phytophthora parasitica* var. *nicotianae* Tucker, induces modification in metabolism involving the carbohydrate and nitrogenous constituents of the leaves of tobacco.

HISTORICAL

Apparently rather few comparative biochemical studies of plants in disease and in health have been made. The several studies of this kind which have come to light from a search of the literature deal with diseases caused by certain viruses, mineral deficiency diseases, and a few others caused by fungi.

As regards virus diseases, Bunzel (1913) established that the sugar content was reduced but the nitrogen content remained unchanged in curly-top-affected sugar beets. Jodidi *et al.* (1918) found, however, that the nitrogen content of spinach was reduced in plants having spinach blight. Brewer *et al.* (1926) reported that the carbohydrate content was reduced in foliage of mosaic-affected tomatoes but that the nitrogen content remained unchanged. Similar modifications were shown by Caldwell (1934) to be induced in tomatoes by the aucuba or yellow

[1] We are grateful to the Research Laboratories, Liggett and Myers Tobacco Company, for making these analyses.

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mosaic virus. Stanley (1937) anlyzed leaves of Turkish tobacco plants inoculated with one or another of several viruses. He found that protein metabolism was increased in plants infected either with ordinary tobacco mosaic virus or with aucuba mosaic virus. But the virus of green cucumber mosaic, of yellow cucumber mosaic, of tobacco etch, and of tobacco ring spot, each induced a decrease in total nitrogen.

Iljin (1951a, 1951b) studied lime-induced chlorosis of nearly a score of herbaceous and woody species by analyzing chlorotic leaves as compared with healthy ones. In general, he found that the nitrogenous constituents, sugars, and acids in chlorotic leaves exceeded those in normal ones, indicating profound disturbances in metabolism. He showed, moreover, that 94 to 96% of the total nitrogen content of mature healthy leaves was protein nitrogen whereas only 30 to 40% of the nitrogen content of chlorotic leaves was protein.

Representative relevant findings with fungus diseases are typified by those which follow. Mains (1917) noted that starch does not accumulate near rust pustules in the parenchyma sheaths of corn, for the reason that it is presumed to have been utilized by the rust fungus itself. Similarly, Orton and Kern (1919) reported that starch is lacking in potato tubers in tissues occupied by the wart fungus, *Synchytrium endobioticum* (Schilb.) Perc. Coons and Klotz (1925) found that the nitrogen content of celery leaves in two diseases caused by the leafspot fungi *Cercospora apii* Fres. and *Septoria apii* Chester, was 61% and 83%, respectively, of that of normal leaves. Hurd and Hasselbring (1927) showed that corn smut, *Ustilago zeae* (Beckm.) Unger, lowers the sugar content of nearby internode tissues to one-sixth that of normal. Kourssanow (1928) recorded a reduction of 20 to 30% in carbohydrate production as a result of wheat smut, *Ustilago tritici* (Pers.) Jens. Schmidt (1932) stated that sugar beet rust, *Uromyces betae* Pers., causes the elaboration of large amounts of fats, instead of starch and sugar, in diseased leaves.

MATERIALS AND METHODS

Samples of healthy and diseased leaves of the varieties Hicks and Gold Dollar were collected from 5 fields. Each sample, composed of one or two leaves from each of 20 to 25 plants, was taken from the middle portion of the stalk. These leaves were of an age to be regarded as mature. As soon as possible after collection, these leaves were attached to sticks in the manner customarily used with flue-cured tobacco and were placed in a barn and cured along with other tobacco.

An additional set of samples was taken from greenhouse-grown Hicks plants. These plants were approximately 16 inches tall, some of them healthy and the others artificially inoculated. All of the leaves, although immature, were removed from these plants at the same time and were divided arbitrarily into three lots, lower, middle, and upper leaves. Then all were dried as rapidly as possible in an oven maintained at approximately 105° Fahr.

All samples were stored at laboratory temperatures for a period of about 5 months before being analyzed. In preparation for the analyses the samples were pulverized in a ball mill, after which weighed aliquots were analyzed. The meth-

ods used for determination of carbohydrates and nitrogenous constituents were those outlined by the Association of Official Agricultural Chemists. Percentages of constituents were calculated, using dry weight of samples as the basis for calculation.

RESULTS

Analytic data from the samples of field-grown leaves are shown graphically in Figs. 1 and 2. The striking feature portrayed by these histograms is that both the carbohydrate metabolism and the nitrogen metabolism are greatly disturbed in affected plants. The elaboration of sugars in diseased leaves is markedly decreased. As is apparent in Fig. 1, the content of total sugars and that of reducing

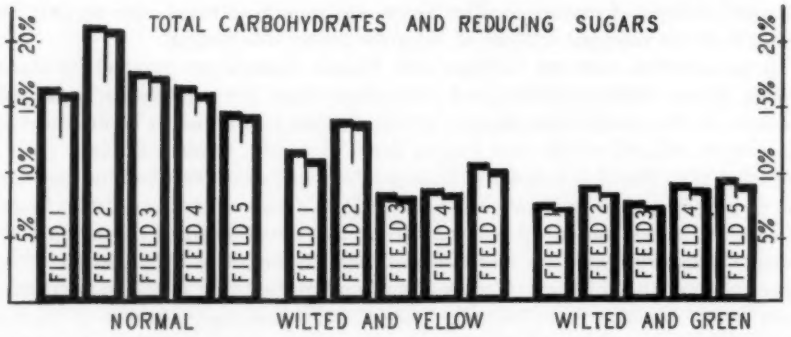

FIG. 1. Histograms showing total sugars and reducing sugars in healthy and black shank-affected leaves of field-grown tobacco. The left half of each column represents percentage content of total sugars, and the right half, reducing sugars.

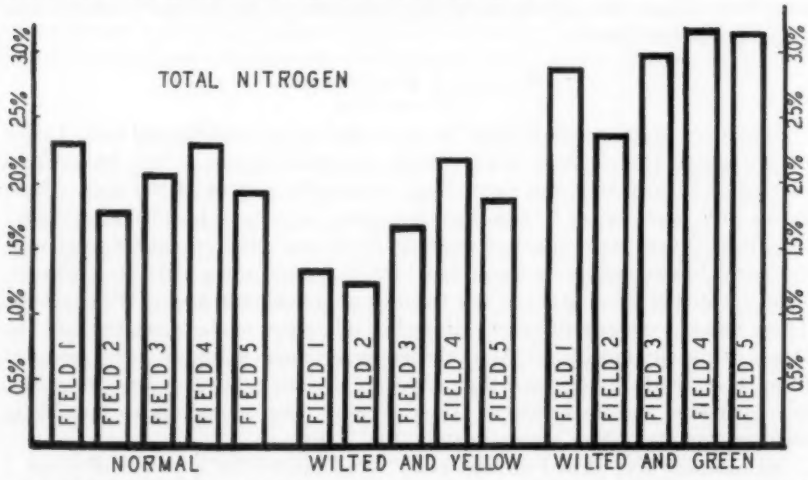

FIG. 2. Histogram showing total nitrogen content of normal and black shank-affected leaves of field-grown tobacco.

TABLE 1

Comparative analyses of leaves of healthy and diseased greenhouse-grown Hicks tobacco

Stalk Position	Constituents							
	Total nitrogen, %		Protein nitrogen, %		Total sugars, %		Reducing sugars, %	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Lower	1.69	2.63	1.06	1.11	6.29	3.50	6.11	3.46
Middle	2.83	3.51	2.04	1.82	6.82	5.70	6.64	5.50
Upper	4.40	3.18	3.11	2.67	9.09	6.60	8.56	5.95
Weighted Average	2.68	3.16	1.85	1.82	7.10	5.23	6.84	4.96

sugars in all samples consistently differ little, one from the other. The average total sugar content of healthy leaves from the 5 fields was 17.18% and the average content of reducing sugars, 16.87%. The total nitrogen content was 2.06%. In contrast, the total sugars and reducing sugars in the diseased, wilted, yellow leaves averaged 10.81% and 10.55%, respectively, and the total nitrogen averaged 1.67%; and the content of total sugars and reducing sugars in the diseased, wilted, green leaves averaged 8.82% and 8.37%, respectively, the total nitrogen averaging 2.92%. It thus appears that there is no direct correlation between modification in carbohydrate synthesis and that involving the synthesis of nitrogenous materials, although protein synthesis is well established to involve materials derived from carbohydrates.

The comparative analyses of leaves of immature plants, grown in the greenhouse, are presented in Table 1.

As in the case of field-grown plants, it is apparent that the black shank pathogen induces a similarly profound disturbance in the elaboration of carbohydrates and proteins. The weighted-average data show that the total sugar content of the leaves from diseased plants is approximately 74% of that of healthy ones, but the proportions of total sugars to reducing sugars in healthy leaves and in diseased leaves are so nearly alike that the differences are not deemed significant.

As regards total nitrogn content of greenhouse-grown plants, it may be seen that the weighted-average content in leaves of diseased plants is greater than that of healthy plants. This fact appears to be less consequential, however, than the fact that the protein nitrogen in leaves of healthy plants accounts for 65% of the total nitrogen content whereas that in leaves of diseased plants is 54.5% of the total nitrogen content. Such results clearly indicate interference with the transformation of soluble nitrogen to proteins.

DISCUSSION

Any interpretation of the results of the foregoing analyses should take into consideration the fact that the pathogen itself occupies the roots and basal

portions of the stems and hence is remote from the leaves, the organs analyzed. Once this fact is appreciated, interest is focused immediately on the problem of the mechanism of induction and of interference with metabolic activities. Also one becomes concerned with how disturbances of such great magnitude could occur in parts so remote, as are the leaves.

Explanation may be presumed to require that account should be taken of the fact that disturbance in the manufacture of sugars and proteins is accompanied by a decrease in water loss in diseased plants (Schramm and Wolf, 1954). But reduction in the amount of water, *per se*, available to the leaf tissues would not be expected to induce such marked modifications in metabolic activities. Consideration might also be given to the fact that there occurs modification in respiratory rate in diseased tissues (Wolf and Schramm, 1954). Such occurrence indicates the probability that respiratory rate and changes involving the elaboration and accumulation of sugars and proteins may be interrelated and interdependent to a degree.

If, ultimately, a causal interrelation should be established to exist between decrease in transpiration and increase in respiratory rate, and, in turn, such changes were found to be related to interference with the processes involved in synthesis of sugars and proteins, the proximate cause for these interrelated modifications in metabolic activities would still remain without adequate explanation. However, in the light of the fact that in disease the vascular elements are occluded, one may postulate, as a proximate cause, the interference of passage from the roots to the leaves of growth-regulating principles produced within the roots. Additionally, it may be postulated that toxins elaborated within the necrotized tissues could be a proximate cause of the black shank syndrome. Probably toxin is produced continuously, and even though it be in small amount, the cumulative effects could be expressed as profound changes in metabolism. Admittedly, too little is yet known about the pathology of black shank-affected leaf tissues to account for the mechanism by which interference in elaboration of sugars and proteins is accomplished.

SUMMARY

Comparative chemical analyses were made of mature leaves of black shank-affected tobacco and of normal leaves grown in the field. Greenhouse-grown leaves were similarly analyzed. These analyses showed that, as a consequence of attack by the black shank fungus, carbohydrate synthesis and protein synthesis are profoundly modified. Leaves from diseased plants have a lesser content of total sugars and reducing sugars than leaves from healthy plants. Also, a larger proportion of the total nitrogen content of leaves from diseased plants is soluble than that of healthy leaves, thus indicating modification of the processes involved in protein elaboration. It is suggested that the mechanism of such modifications or the proximate cause may be related to reactions involving toxins.

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AN INTERPRETATION: SEXUALITY OF FUNGI

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As a consequence of interest among mycologists in sexual phenomena among fungi there has come into being a vast literature, and from perusal of even a small fragment of these studies one is forced to conclude that wide differences in terminology, interpretation, and conclusion exist. In the opinion of one internationally known cytogeneticist a state of hopeless confusion exists in the field of sexuality among fungi. The writer is well aware of the fact that any effort to harmonize or to unify these differences may be fraught with far-reaching consequences, not the least of which is the jeopardy in which one places his most prized possession, his standing among fellow scientists. Nevertheless an interpretation quite at variance with widely accepted ones is being made, but only after considered deliberation as to the consequences and with the earnest hope that confusion will be somewhat decreased thereby, not increased.

This interpretation is an outgrowth of attempts to answer students' questions, many of which appear to be unrelated but the answers for which may ultimately be found to show that all are related and that appropriate answers may depend upon establishment of basic concepts of sexuality. Among such questions are the following: (1) What is the origin of sexuality among fungi? (2) Are there several distinct origins of sexuality corresponding with the evolution of the different groups of fungi? (3) Account for the conclusion that within a closely related group of fungi some are sexual whereas others are only asexual. (4) If sex is dual and is divided at meiosis how can a fungus be homothallic? (5) If one accepts the conclusion that homothallism and heterothallism are basic phenomena how is it that some of the species among certain genera or closely related genera are homothallic whereas others are heterothallic? (6) Granted that homothallism and heterothallism are fundamental, account for the fact that certain species, such as *Pleurage anserina*, exhibit both phenomena. (7) If the sex of a given spore of a heterothallic species is determined by the time that the spore is delimited how can the thallus arising from that spore react as a (+) strain in one mating and as a (−) strain in the other mating? (8) By the development of what structure or occurrence of what process does a fungus become asexual? (10) If conidia are asexual how can they function as spermatia, as in *Neurospora sitophila*? (11) Explain how sex can be genotypic in one species and phenotypic in another. (12) How is it possible for a female gamete, without fusion, to develop into a thallus bearing only antheridia? (13) Account for the incidence of conjugate nuclear division. (14) The dicaryotic state is maintained by repeated conjugate division among Hymenomycetes. What is the significance of such occurrence? (15) Why is no particular significance attached to hyphal fusion among Deuteromycetes? (16) Do any species of fungi possess "sex chromosomes"? (17) If there is no evidence of "sex chromosomes" on the basis of chromosome morphology, why have sex chromosomes not been postulated to occur on the basis of genetic evidence?

Satisfactory answers to the foregoing questions, and to many similar ones that are equally pertinent which have been asked of me, are not found in mycological literature. Admittedly, answers of a sort exist but mostly as restatements of the problems involved. For this reason an interpretation of sexuality is herein proposed merely as another attempt to provide a basis from which answers to queries of this kind may be approximated.

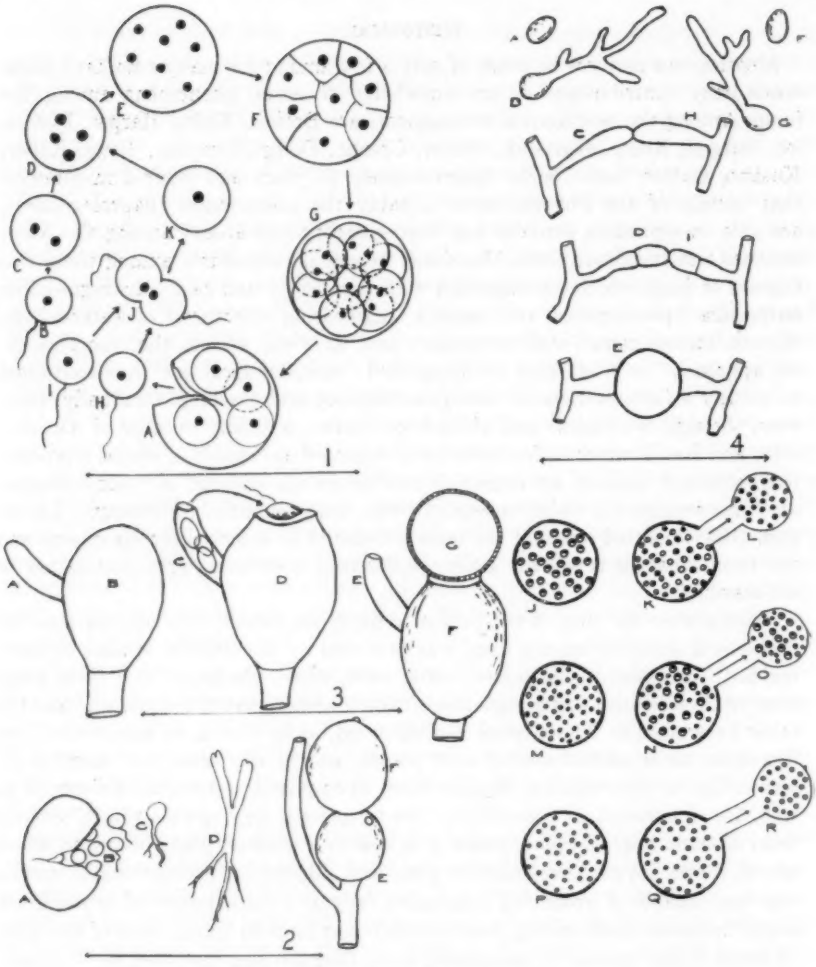
To do so, a body of evidence will be presented to indicate that all fungi may be alike as to the fundamental nature of their sexuality. This will be done by consideration of a few representative species selected from among the three Classes, Phycomycetes, Ascomycetes, and Basidiomycetes. Those selected are by no means exceptions. The essential observations will be taken largely from the publications of the investigators cited throughout this report. It is hoped that confusion revolving around matters of accepted terminology, which this approach entails, will in itself not present insurmountable difficulties nor create further misunderstandings. At least, by so doing, the coinage of new terms is avoided.

HISTORICAL

Mention can perforce be made of only a few among the many who have made noteworthy contributions to our knowledge of sexual phenomena among the fungi. Among the best-known investigators are Brefeld, Klebs, Harper, Blakeslee, Burgeff, Kniep, Bensaude, Buller, Craigie, Dodge, Drayton, Ames, Raper, Kusano, Cutter, and Couch. Approximately 75 years ago Brefeld maintained that certain of the Phycomycetes, notably the water-molds (Saprolegniales), are able to reproduce sexually but that sexuality was absent among the Ascomycetes and Basidiomycetes. Manifestly he saw no structures among these two Classes of fungi which corresponded with the clearly and easily distinguishable antheridia (spermogonia) and oogonia (egg-bearing structures) of water-molds. His conclusions gained wide acceptance and, as is well known, the "morphological approach" or what may be designated "morphological sex" has continued to occupy a favored niche in biological thinking and teaching. Gradually, however, through cytological and life-history studies, a goodly number of Ascomycetes and Basidiomycetes has come to be regarded as capable of sexual reproduction, although lacking sex organs. Nevertheless the concept of "morphological sex" has remained a widely-accepted credo. Only recently, for example, Lindegren (1948) pointed out that the term sex should be reserved for sex organs and the term "mating type" for genetic differences controlling cross-copulation or self-sterility.

Shortly after the turn of the present century an entirely new approach to the problem of sexuality among fungi was provided by the classical studies of black molds (Mucorales) by Blakeslee (1904, 1906, 1920). He found that these fungi may be divided into two groups one of which he designated homothallic and the other heterothallic. These terms corresponded, as he stated, to monoecious and dioecious, as employed among seed plants, and in this sense are morphologic terms. But he later came to employ them to emphasize functional differences as well. He designated as homothallic (sex-totipotent) any species which, starting from a single haploid spore, could give rise to a thallus (plant body) on which sexual spores (zygospores) may be produced. Moreover, a heterothallic species was one capable of producing zygospores only as a consequence of sexual interaction between thalli arising from two different haploid spores. One of the thalli of heterothallic species he designated as + (female) and the other as − (male).

Subsequently the phenomenon of heterothallism has been ascribed to many other species of fungi occurring in widely separated groups. An idea of such incidence of heterothallism can be gained from a summary by Kniep (1928). He reported that only one among 94 species of Saprolegniales has been found heterothallic and 24 homothallic, and that 51 species of Laboulbeniales are known to be heterothallic and 11 species, homothallic. Among Ascomycetes, other than the Laboulbeniales, a fair proportion was found to be homothallic. Among Ustilaginales (smut fungi), 19 had been proved to be heterothallic and 2 homothallic. Among the Uredinales (rust fungi), 19 had been shown to be heterothallic and 4 to be homothallic. Approximately 90 percent of the 230 species of Hyme-



FIGS. 1–4

FIG. 1. *Olpidium*. A, Opened "sporangium" emitting uninucleate planospores; B, A single uniciliate motile spore (planospore); C, The cilium has been retracted and the cell has grown; D, The thallus has increased further and the nuclei have increased in number; E, The thallus immediately prior to cleavage; F, Cleavage of the thallus; G, The segments have rounded to become spores; H & I, A pair of planospores of the same origin and potential as B; J, Cellular fusion of H and I after which the nuclei will fuse and meiosis will follow to give a thallus like D; K, The zygote has formed. FIG. 2. *Allomyces arbusculus*. A, Sporangium emitting motile spores; B, Uniciliate spores; C, Young plant soon after spore germination; D, A later stage; E & F, Branch of plant, the upper globular cell, F, bears several egg-cells, the lower, E, sperm-cells. FIG. 3. *Monoblepharis polymorpha*. A & B, Hyphal tip which becomes the oogonium, B, bearing a lateral branch which becomes the spermogonium,

nomycetes (mostly gill fungi or mushrooms) and Gastromycetes (mostly puffballs) examined had been found heterothallic.

Subsequently to Kniep's report, many other species have been found to be heterothallic but the great majority remain to be investigated. But in determining whether any particular species is heterothallic, different meanings of the term have been employed for differences in mating structures and mating behavior with consequent resultant confusion. Whitehouse (1949, 1951) would group heterothallic fungi into those which are (a) "morphologically heterothallic", and (b) those which are "physiologically heterothallic". This terminology does not clarify matters because morphologically heterothallic species are themselves physiologically heterothallic. Drayton and Groves (1952) and certain other mycologists continue to interpret the words homothallic and heterothallic as morphological terms. Korf (1952) stated that these two terms were coined by Blakeslee to denote a physiological phenomenon, not a morphological one.

SEXUALITY AMONG PHYCOMYCETES

Brief consideration will be given, in turn, to certain Chytridiales, Blastocladiales, Monoblepharidales, Saprolegniales, and Mucorales.

CHYTRIDIALES.—The researches of Kusano (1912) on *Olpidium viciae* (Fig. 1, A–J) and on *Synchytrium fulgens* (Kusano, 1930), and of Curtis (1921) on *Synchytrium endobioticum* may be interpreted to show that these species have in common the characteristic feature of producing "angia" (sporangia?, gametangia?) within which uninucleate planospores (motile spores) are delimited by cleavage. The planospores arising within any given "angium" may reasonably be expected to be identical as regards nuclear constitution, yet certain of these planospores can behave as asexual spores and others can unite by twos, one acting as a sperm-cell and the other as an egg-cell. By virtue of similar origin each motile spore may therefore be assumed to be similarly totipotent. Hence, if one designates the sexual factors as (+) and (−), each spore must possess both (+) and (−) and it is therefore bisexual or hermaphroditic, using these words in the accepted sense. Whether any given planospore behaves asexually or whether fusion of pairs occurs might conceivably be related, as one condition, to imbalance between (+) and (−). Such imbalance may have existed when the spores were first formed or it may have arisen subsequently in response to

A; C & D, A later stage having a motile sperm-cell from C lodged at the receptive papilla of the oogonium, D; E, F & G, The fertilized egg-cell, A, attached on the empty oogonium, F, has become thick-walled. E, empty spermogonium. FIG. 4. *Mucorales*. A & F, (+) and (−) sporangiospores of *Mucor mucedo*, as commonly understood; B & G, Young (+) and (−) thalli; C & H, Progametangia; D & I, Gametangia delimited from tips of progametangia; E, Zygospore resulting from fusion of gametangia; J, Fused nuclei in zygospores of *Mucor mucedo;* K, Germinating zygospores of *M. mucedo;* L, Nuclei in germ sporangium all of one potential; M, Mature zygospore of *Phycomyces blakesleeanus* containing non-fused nuclei; N, Not all complementary nuclei have fused when the zygospore germinates; O, Non-fused and fused nuclei within the germ sporangium; P, Mature zygospores of *Sporodinia grandis* containing non-fused nuclei; Q, Germinating zygospores containing non-fused nuclei; R, Germ sporangium containing non-fused nuclei.

external stimuli. It would follow that if the two factors were in balance the spore would be neutral, and would therefore lack ability to fuse. If, on the other hand, imbalance existed or could be induced by mutual interaction of a pair of motile spores, then fusion might occur.

BLASTOCLADIALES.—In Fig. 2, E & F, is shown a portion of the thallus of *Allomyces arbusculus* bearing a fertile hyphal branch. The terminal cell, F, constitutes the oogonium (an egg-cell-bearing structure) and the cell immediately beneath it is the spermogonium (a sperm-cell-bearing structure, E). These two elements commonly have the same arrangement in *A. javanicus* but sometimes the positions are reversed. The oogonium produces several egg-cells, and the spermogonium several sperm-cells. At a certain developmental stage the septum to delimit the oogonium from the spermogonium has not yet formed. It is usually assumed that only the factor for (+) exists in the nuclei of the oogonium and that for (−) in those of the spermogonium. But the migration of these two kinds of nuclei in opposite directions prior to the formation of the septum, if such migration does occur, is not known to take place. Rather than maintain that there is migration it seems reasonable to assume that the nuclei are all alike, that each contains (+−), and that imbalance between egg-cell nuclei and sperm-cell nuclei is induced, after the septum has been formed.

The observations by Emerson (1950) on *Blastocladiella variabilis* are especially noteworthy in an interpretation of sexual behavior among Blastocladiales. In this species the antheridia and oogonia are borne on different thalli (plants), i.e. the species is dioecious. But female gametes may germinate parthenogenetically and develop into plants some of which bear only antheridia, others only oogonia. To explain this phenomenon it is stated that there is no segregation of male and female determiners at meiosis. Could this mean that the female gamete is not female, but instead is totipotent?

MONOBLEPHARIDALES.—A portion of the thallus of *Monoblepharis polymorpha* is shown in Fig. 3, A–G, to indicate stages in development of the oogonium and spermogonium. The spermogonium, A, is borne upon the oogonium, B, an arrangement opposite from that in *Allomyces arbusculus*. How "male" nuclei are separated from and arise from "female" nuclei remains unexplained. So again as in *Allomyces arbusculus* it is suggested that "male" nuclei may be hermaphroditic but become predominantly (−), and "female" nuclei likewise may be hermaphroditic but become predominantly (+) by differentiation after the oogonium and spermogonium have been delimited.

SAPROLEGNIALES.—From studies of the water mold, *Sapromyces elongatus*, Bishop (1940) isolated strains which he interpreted to be of the following kinds (1) pure male; (2) pure female, which remained sterile when grown separately, but which produced antheridia and oogonia when grown in paired cultures; (3) typical self-sterile hermaphrodites; (4) neutrals, which were found incapable of forming antheridia and oogonia; (5) females, which when mated with pure females, responded in such a way as to be interpreted as having latent maleness; (6) males which when mated with pure males responded in such a way as to be interpreted as having latent femaleness. These interpretations of relative sexu-

ality are not unlike those from mating reactions among fruit-flies. These interpretations assume that maleness may be represented by MM and Mm and femaleness by FF and Ff. On this basis Bishop interpreted the six strains to be either Mf, mF, or FF, and that there could be found to exist in addition MM, MF, and mf strains. Instead, could it be that the mating reactions are merely evidences of degrees of imbalance in potential?

The studies by Raper (1940, 1950) of certain species of *Achlya* have special relevance to the present interpretation. He found when he grew the heterothallic water mold, *Achlya bisexualis*, having the (+) isolate in one water culture and the (−) isolate in another, that the oogonial and spermogonial branches failed to develop. But if he reciprocally transferred a small quantity of water from one culture to the other, spermogonia were initiated in the culture to which water from the (+) culture had been added, and oogonia were initiated in the culture to which water from the (−) culture had been added. Furthermore the spermogonia and oogonia failed to mature unless another reciprocal transfer of water was made subsequent to formation of spermogonial and oogonial initials. This evidence led him to conclude that at least 2 hormones are secreted by the (+) culture which are essential for the development and maturation of spermogonia, and that at least 2 other hormones are secreted by the (−) culture which are essential for the development and maturation of the oogonia. Differentiation of the sexual structures was thus concluded to be conditioned by the reciprocal mechanism of hormone-induced reactions.

Subsequently Raper (1950) attempted to hybridize several isolates of *Achlya americana*, a homothallic species, with *A. bisexualis*. He used both the (+) strain and the (−) strain of *A. bisexualis* in these trials, and found that mature oospores were formed with certain isolates of *A. americana* when crossed with the (+) strain of A. *bisexualis*. With other isolates of *A. americana*, however, mature oospores formed only when crossed with the (−) strain of *A. bisexualis*. With other isolates of *A. americana*, spermogonia, or else oogonia, were initiated when the thalli were mated, but all further development of sexual structures ceased. With still other isolates of *A. americana*, there was no evidence of interaction when either the (+) strain or the (−) strain of *A. bisexualis* was used in the matings. Could these "relative sexuality" differences in reaction exhibited by the different strains of *A. americana* be explained if the nuclei of *A. americana* are assumed to be totipotent, and that the differences involve imbalance of (+) and (−) factors? If (+) and (−) were in balance then the strain of *A. americana* would be neutral to both strains of *A. bisexualis*. If the thalli of *A. americana* were preponderantly (+), then they would mate with the (−) strain of *A. bisexualis*, only if the latter were preponderantly (−), and vice versa.

The interesting feature in these foregoing studies with water molds is response to "action-at-a-distance". Such results suggest that a similar hormonal basis for induction of sexual differentiation may one day be demonstrated to occur in many other species of fungi.

MUCORALES.—As previously indicated, the researches with the black molds by Blakeslee mark the beginning of studies of heterothallism, i.e. they involve

fusion between gametangia (gamete-containing structures) of two differing thalli, a (+) thallus and a (−) thallus. Blakeslee (1904) found that such a condition obtains with *Mucor mucedo*. As shown in Fig. 4, A–I, when monosporic cultures, originating from sporangiospores, A and F, of this fungus, are planted at some distance from each other in the same culture dish, certain opposed hyphae from the two colonies, as they approximate each other, form pairs of club-shaped hyphal tips, called progametangia, C and H. The tip of each progametangium is next delimited by a septum, thus forming the pair of complementary gametangia, D and I. The walls at the point of contact between the paired gametangia are next dissolved and the gametangial contents fuse to form the zygote (fertilized egg-cell), E. Then a thick resistant envelope is formed surrounding the zygote which sooner or later is modified to become the zygospore.

When Blakeslee (1920) subsequently attempted to determine the mating potential of a large number of isolates of *Mucor mucedo* he noted that some of them behaved as (+) races, others as (−) races, and certain others failed completely to react in the presence of the (+) race or of the (−) race. He regarded these latter ones as "neutrals", and interestingly observed that neutrality could be induced by growth at high temperatures.

When zygospores of Mucorales are germinated, Blakeslee (1906) found that a germ tube is formed from each one, which is terminated by a germ sporangium (Fig. 4, J–R). Moreover he found that the spores produced in any given germ sporangium of *Mucor mucedo* are all of the (+) potential whereas those in another germ sporangium of this species might all be of the (−) potential (Fig. 4, L). In *Phycomyces blakesleeanus*, as verified by the cytological findings of Cutter (1942), part of the spores within a given germ sporangium are (+), others (−), and still others (+−) (Fig. 4, O). This phenomenon was found to be due to failure of fusion of certain of the nuclei and also failure of meiosis to involve all of those that had fused.

In the homothallic *Sporodinia grandis* each spore from the germ sporangium was found to be homothallic (sex-totipotent). The cytologic evidence by Cutter (1942) shows that nuclear fusions and meiotic division do not occur in this species during formation of zygospores or their germination (Fig. 4, P–R). In explanation he stated that "apomictic development of *Sporodinia* apparently represents the loss of sexual function of the nuclei with physiological differences in the plasma, under the influence of external conditions sufficient to produce a pseudosexual reproductive mechanism."

Perhaps the divergence in patterns of sex-differentiation by the species of Mucorales, above mentioned, is susceptible to explanation in a way more closely to relate them. As Cutter (1942) found, nuclear fusions, involving union in pairs of nuclei having (+) potential with those having (−) potential, occurs at the time of gametic conjugations, and meiotic division occurs soon thereafter in *Mucor mucedo*. But, strangely, the spores within any one germ sporangium proved, by mating trials, to be all of one sex-potential. This poses the problem of the fate of the nuclei of the complementary sex-potential, a problem which to date appears to have remained unsolved. Sjöwall's explanation (1945) in-

volves nuclear degeneration, during the resting period, of all nuclei of the same potential in one zygospore and of all those of the other sexual phase in another zygospore. This explanation has been deemed satisfactory and, in spite of its inherent strangeness, has gained a goodly measure of acceptance. It is possible that all of the spores (and their nuclei) in any germ sporangium of *Mucor mucedo* may be totipotent regardless of whether their mating responses indicated them to be either all (+) or else all (−)? If this be true, there may have arisen sufficient imbalance between (+) and (−) to make them all react as (+), or else all as (−). Such imbalance could have been induced by hormonal activity.

The failure of paired fusion to involve all nuclei in *Phycomyces blakesleeanus* may not be unlike findings in *Cunninghamella* since its mating behavior (Burger, 1919) follows a similar pattern. Burger isolated a (+) race, herein designated A, and a (−) race, herein designated B. He then isolated a race, C, which mated with A, and which therefore must have been a (−) race. Then he mated "mycelia of the same sex" (B and C) with the production of normal zygospores. Burger did not explain this phenomenon but it appears highly probable that each strain was totipotent, differing only in degree of imbalance of factors for mating potential.

The homothallic *Sporodinia grandis* presents a special situation, one in which differentiation of the gametangia proceeds only to the stage that gametangia are matured, the walls in contact are dissolved and the gametangial contents are intermingled, indicating the existence of some measure of imbalance. But the magnitude of imbalance is of such low order as not to induce nuclear fusion. Even without nuclear fusion it is conceivable that the totipotent nuclei contributed by one gametangium may have exerted a "sexual" influence upon the totipotent nuclei from the other gametangium, and that this process thus constitutes an "imperfect" or "incomplete" fertilization yet, in effect, fertilization but of a very reduced kind.

SEXUALITY AMONG ASCOMYCETES

For the sake of brevity, primarily, consideration will be given only to a few ascomycetous fungi.

YEAST.—Two mating types have been shown by Lindegren (1948) to occur in the common yeast, *Saccharomyces cerevisiae*. The ascus of this yeast typically contains 4 ascospores, two of which he designated a type and the other two α type. Normally a cell (gamete) of the a type must be mated with one of the α type but he noted that occasionally fertile matings, which he called illegitimate matings, occurred between cells of the same mating type. Apparently these results, both legitimate and illegitimate matings, may be explained by assuming that each gamete contains both of the factors a and α, with a preponderance of a in one gamete and of α in the complementary gamete, to account for mating potential.

PINK BAKERY MOLD.—The pink bakery mold, *Neurospora sitophila*, and related species, have been used extensively in genetical investigations. By removing each of the 8 ascospores seriatim (i.e. as to position in the ascus) from

an ascus, then by growing each one separately in pure culture and mating the eight cultures in all possible paired combinations, Wilcox (1928) found that there are two mating types. Dodge (1927, 1935) regarded this organism as heterothallic and designated the spore groups or types as A and B. Sequential stages in the formation of uninucleate ascospores of the two types are shown in Fig. 5, A–E. Subsequent studies by Dodge (1940) and Backus (1939) established the highly significant fact that any of the monosporic isolates, whether of the A type (Fig. 5, G–K) or of the B type (Fig. 5, M–P) produced colonies

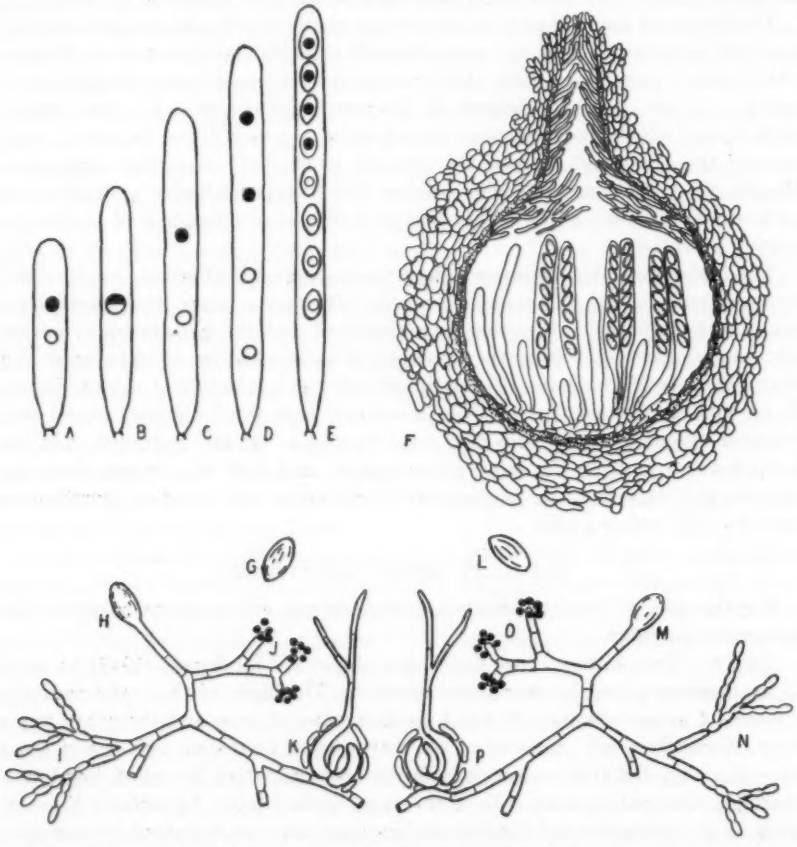


FIG. 5. *Neurospora sitophila*. A, Young hyphal tip which is to become an ascus, containing two nuclei of different mating potential: B, Fused nuclei of the primary-ascus nucleus; C & D, Products of meiosis; E, Eight-spored ascus having spores of two mating types; F, Vertical section through mature perithecium; G & L, Ascospores of complementary mating potential; H, I, J & K, Germinating ascospore, conidia, spermatia, ascogonium, respectively (thallus of one mating potential); M, N, O & P, Germinating ascospore, conidia, spermatia, and ascogonium, respectively (thallus of one mating potential).

having spermatia-producing branches (male sex structures), sclerotial masses containing ascogonia (female sex structures), and also conidia of the form genus *Monilia*. Equally important is the fact that perithecia were never developed in monosporic cultures even though both sex structures were formed. But if spermatia from type A cultures were applied to the branched trichogynes (receptive portions of the ascogonia) of type B cultures or vice versa, then perithecial development followed and perithecial maturity was soon attained. Each monosporic culture was thus shown to be self-sterile, but there was cross-fertility or cross-compatibility between the two types. What at first sight may be more remarkable, as determined by Dodge and Backus, was the fact that germinating ascospores, or else conidia, or even bits of mycelium taken prior to the production of conidia, when similarly applied reciprocally, also induced the development of perithecia. This phenomenon was interpreted as "substitute sexuality". It is therefore apparent that each cell throughout the entire thallus originating from an A type spore and similarly that from a B type spore, is sexually totipotent. The factor or factors making possible the induction of compatibility however, are separated at meiosis. Such imbalance has been appropriately designated self-sterility, but becomes merely an expression of imbalance in mating potential.

OTHER ASCOMYCETES.—The results of observations on certain other pyrenomycetous species could be employed to provide for similarly relevant interpretations. These include *Neurospora tetrasperma* (Dodge, 1935), *Pleurage anserina* (Ames, 1934), *Bombardia lunata* (Zickler, 1934), *Venturia inaequalis* (Keitt, 1952), *Chromocrea spinulosa* (Mathieson, 1952) and also several discomycetous species (Drayton and Groves, 1952), in the Sclerotiniaceae.

As regards *Neurospora tetrasperma* and *Pleurage anserina*, each is known to produce 4-spored asci, normally. Each ascospore is generally binucleate and furthermore from each spore a mycelium capable of forming perithecia may arise. Each species is therefore deemed homothallic. Occasionally, however, uninucleate ascospores are formed. When such uninucleate ascospores are isolated in monosporic cultures, spermogonia and ascogonia develop on the mycelium, but perithecia are not induced to be formed unless spermatia are reciprocally transferred from a different monosporic culture. The thalli from cultures of uninucleate spores are thus shown to be self-sterile but are cross-compatible and so have come to be regarded as heterothallic.

Apparently these two fungi present the anomalous situation of being both homothallic and heterothallic. This situation appears resolvable if one interprets the presence of spermogonia and ascogonia on the same thallus, whether originating either from binucleate or uninucleate ascospores, as evidence of hermaphroditism. In such case, the factor or factors which induce compatibility or mating potential, are separated at meiotic division.

The mating reactions of three species of closely related disc fungi have been studied (Drayton and Groves, 1952) with the following results. *Stromatinia narcissi* requires two distinct thalli, one "male", and the other "female" and is therefore heterothallic, as first defined by Blakeslee. *Sclerotinia sclerotiorum* is, by the same token, homothallic, since a single ascospore may give rise to a

thallus bearing spermatia and ascogonia which are capable of effecting fertilization. On the other hand, *Stromatinia gladioli* produces both spermatia and ascogonia on the thallus arising from a single ascospore, but the thallus is not self-fertile. The spores of this species are found to be of two mating groups as regards compatibility.

In keeping with interpretations of sexuality among all the other fungi, previously discussed, these disc fungi are herein deemed to differ only in mating-potential, as conditioned by imbalance. In *Stromatinia narcissi*, one entire type thallus is strongly (+) and weakly (−), and the other is strongly (−) and weakly (+). In *Sclerotinia sclerotiorum* the potential differences in balance involve the spermatia and the ascogonia, and imbalance between (+) and (−) is made obvious during the differentiation of the sexual structures. In *Stromatinia gladioli* each thallus is in reality (+−) throughout, but one behaves as (+) and the other as (−), dependent presumably upon hormonally-induced responses in the presence of the complementary mating type.

SEXUALITY AMONG BASIDIOMYCETES

The results of investigations of basidium-bearing fungi, the mushrooms or toadstools being the species most commonly studied, show that a large proportion are heterothallic. The basidiospores of such fungi are typically uninucleate. Similarly all of the cells constituting the primary mycelium, i.e. those cells which arise from germination of the basidiospores, are uninucleate. However, contact of primary mycelia of complementary sex potential results in hyphal fusion (Fig. 6) and dicaryotization (commonly designated diploidization), and thus initiates the secondary mycelium. By conjugate division of the original pair of nuclei contributed by the fusing cells of the primary mycelia and of their progeny, through the mechanism of clamp-connections, all cells of the secondary mycelium eventually are made dicaryotic. Although clamp-connections have been known to exist among Basidiomycetes since 1856, an understanding of their relation to the mechanism of dicaryotization was first brought to light by the researches of Kniep (1913, 1915, 1916, 1917) and Bensaude (1918), and is shown diagrammatically in Fig. 6.

When a basidium-bearing fungus has developed to the extent of forming pilei (fruiting structures, as of mushrooms) each conjugate pair of nuclei fuses within the hyphal tips which are to become basidia. If one pair of factors is concerned in sexuality, as shown in Fig. 7, (upper series), the quartet of basidiospores normally formed on each basidium will consist of 2 spores having one mating potential (+) and two spores having the complementary mating potential (−). If two mating factors are involved (Fig. 7, lower series), then each member of the quartet will be of different mating potential. Some have interpreted such a phenomenon to indicate the existence of 4 sexes or of tetrapolarity. By this same token, if 3 or more factors are involved, the number of resultant sexes would be increased to 8, 16, or 32, etc.

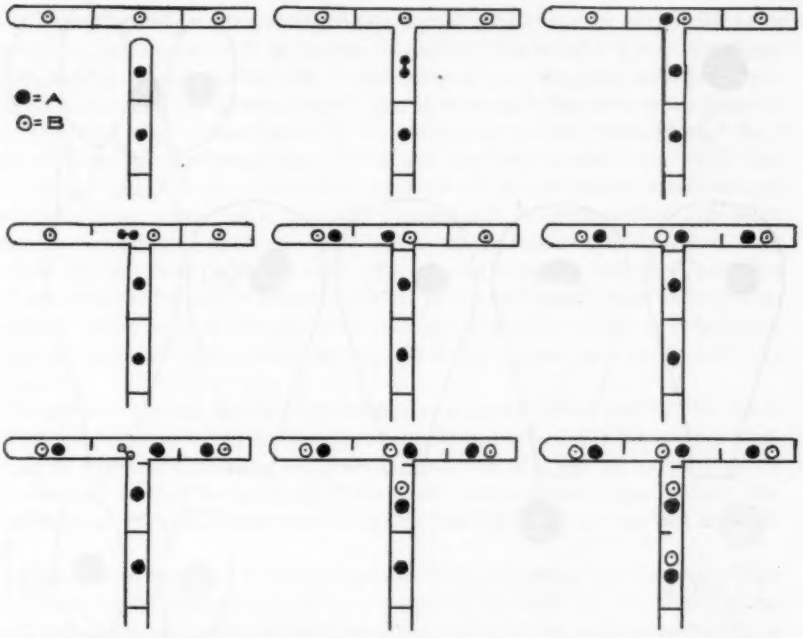


FIG. 6. Basidiomycete diagrams showing hyphal portion of primary mycelia of different mating potential and serial dicaryotization of the cells of these hyphae. The "gates" or incomplete septations make possible nuclear migration. Once the cells are dicaryotized, all new cells arising therefrom have clamp connections. (After Buller.)

If the developmental changes just described, involving basidium-bearing fungi, are typical for this group then at meiosis there occurs segregation of sex factors, according to the commonly-accepted interpretation. But it may again be assumed that each basidiospore remains (+ −). Whether the thalli from any two basidiospores can fuse, and the conjugate-nuclear-pair progeny can ultimately fuse in the young basidium, may be conditioned by imbalance between (+) and (−) in each member of the pair.

No one seems ever to have accounted for the occurrence of repeated conjugate nuclear divisions with an elapsed interval of years, sometimes, between hyphal fusion and association of nuclei in pairs and ultimate nuclear fusion of progeny pairs. Admittedly each member of the pair reciprocally exerts influence upon the other, and the differentiation existing in each nucleus at the time of hyphal fusion is maintained continuously throughout the entire period of conjugate division. Apparently this phenomenon of maintenance of imbalance must depend upon hormonal action, and is as truly sexual in nature as nuclear phenomena occurring among Phycomycetes and Ascomycetes having rather prompt nuclear fusions.

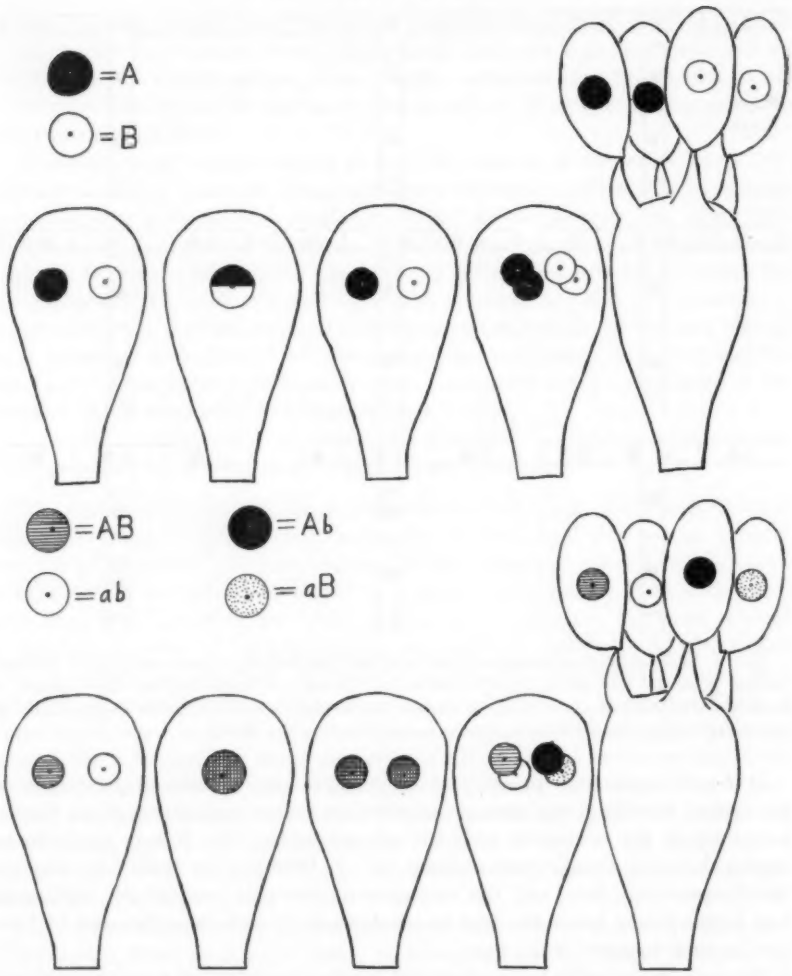


FIG. 7. Basidia in diagram. Upper row, conjugate nuclei in hyphal tip, in which case there is a one-factor difference in mating type between the nuclei. Follows nuclear fusion, meiosis, and production of 4 basidiospores of two types, commonly called bipolar spores. Lower row, a similar sequence except that a pair of factors is involved in the mating, with resultant basidiospores, each of which is different, commonly called tetrapolar spores. (After Buller).

DISCUSSION

It appears pertinent to recount certain essentials which cytologists and geneticists (Muller, 1932, Darlington, 1937, Snyder, 1940, Sinnott, Dunn, and Dobzhansky, 1950) have established regarding sex among animals and seed plants

as a background for interpreting fundamental similarities in sexual behavior among the representative fungi herein considered. The results of such investigations may be placed in either one or the other of two categories as regards sex-differentiation and sex-determination. One of those includes those which support the idea that sex is conditioned by the mechanism of sex chromosomes, by a balance between sex chromosomes and autosomes (any chromosomes other than sex chromosomes), or by interaction of genes of the sex chromosomes and/or autosomes. Such a concept is basically morphologic or mechanistic. The other category comprises those whose experiments emphasize environic factors, such as light, temperature, and nutrition, or such conditions as rate of metabolism and action of hormones or other chemical products as governors of sex-determination. Massive data in support of each interpretation exist but it is appreciated by most biologists that these two concepts are not necessarily mutually exclusive.

All appear to agree that sex-inheritance is gene-controlled and by this token is Mendelian just as is the heritability and transmission of any other characters. All agree that there must normally occur (1) fusion of a pair of complementary cells and (2) meiosis to segregate homologous chromosomes, thus making possible independent assortment and recombination, and also linkage and crossing-over.

Apparently, however, an entirely satisfactory explanation has not been made to account for the differentiation of a complementary pair of "sex" cells having "sex" cytoplasm and "sex" nuclei. Admittedly it involves function as having to do with inbreeding and outbreeding and as related to survival of the species. All available evidence shows that inbreeding tends to be greatest in species which lack any appreciable amount of morphologic differentiation of sex cells. On the other hand, the chances of outbreeding are enhanced in species in which the complementary cells are differentiated morphologically, or in which heterothallism occurs, or in which cross-fertility as opposed to self-sterility occurs, or in those which produce spermogonia and oogonia or other male and female organs. Mather (1940) and also other students of problems of sexuality have emphasized that the greater the concurrent structural differentiation the greater the probability of survival of the species in an ever-changing environment or of adjustment to a variety of habitats.

Unisexuality, as usually defined, exists in relatively few species of seed plants, and such are appropriately designated as dioecious species. It has become differentiated however in most animals. All such unisexual individuals, animal or plant, are spoken of as males if they are able to produce male gametes and as females if they produce female gametes. Abundant evidence of "sex reversal" in both animals and seed plants has accumulated, showing that unisexuality is not to be regarded as a fixed, irreversible state. One may postulate for such phenomena, as has been done, that every individual animal or plant possesses all the genes (complementary sets) necessary for both "sex-potentials", but that the direction of differentiation normally allows one set of genes to function to a greater extent than that of the complementary set. If this be true, the zygote

(fertilized egg-cell) on developing, has the inherent or intrinsic ability, as stated by Snyder (1940) "to react in either of two ways, one resulting in manifestations of one sex, the other in manifestations of the opposite (complementary) sex. Moreover maleness and femaleness are only specific degrees of a potentially continuous series of stages."

Many mycologists have come to believe that a considerable number of Phycomycetes, Ascomycetes, and Basidiomycetes are unisexual. They speak of unisexual thalli, and unisexual spores containing unisexual nuclei, and hence of either male or female thalli, and of either male or female spores containing either male or female nuclei, respectively. The implication from such a concept is that sex and sexual differentiation are basically morphological. Structure and function however must remain distinct, even though too commonly they are regarded as interchangeable. Sex must be thought of as fundamentally functional. It normally manifests itself by a series of catenate interrelated and interdependent stages or processes. These processes include (1) differentiation of gametangia and gametes or their equivalents; (2) cytogamy or plasmogamy, an intermingling of cytoplasms; (3) nuclear association of a complementary pair or of pairs followed soon by karyogamy (nuclear fusion or diploidization). If karyogamy is delayed, as generally occurs in Ascomycetes and Basidiomycetes, a series of conjugate nuclear divisions of the complementary pair and their progeny may intervene prior to karyogamy, and the resulting production of the zygote; (4) meiosis, or division of the diploid zygote nucleus by which process the series of sexual processes is terminated and the organism is returned to the haploid state.

But if sex is interpreted as being expressed by a series of physiologic processes and thus to be functional rather than morphologic then one is immediately confronted with the query how did sex originate, how is it acquired and how is it lost? The point is stressed herein that the sexual process begins with the differentiation of gametes. Actually however, the potential to initiate differentiation must have already been present prior to the beginning of differentiation of gametes. Similarly meiosis is stressed as terminating the series of sexual processes. But this must not be taken to mean that sex is lost as a result of meiosis. In fact there seems to be no evidence from which it may be concluded that sex is ever acquired or ever lost. In the last analysis, sexuality cannot be separated from the concepts "life" and "living". It must therefore be conceived to be an innate, ever-present property of living protoplasms. If this be true, then there cannot be two sexes, but sex must be conceived to be an indivisible unit, an entity having two complementary, not opposite, mating potentials. Then each haploid dividend arising at meiosis must retain sexual totipotency but the direction and amount of differentiation, i.e. the gradient, toward one potential (maleness) or toward the other (femaleness) conditions mating potential.

There are those who would hasten to indicate that this interpretation merely states that haplonts are hermaphroditic. Presumably such persons react in this way because they have been conditioned by a background of "morphologic sex" training to conceive of sex as binary or as containing two opposite and separable elements.

As further explanation of the concept of sex as an indivisible unit it may be suggested that certain analogies may have relevance. For example, the enzyme potential in each dividend arising from meiosis in a given species is like every other, and enzyme potential is not structural but depends upon the presence and activity of genes. Enzyme potential thus becomes an intrinsic property of the specific protoplast.

Again, the measurement of hydrogen ion concentration by the pH scale may be thought of as somewhat analogous to the present concept that sex is a unit with two mating potentials. One extreme of this scale, designated pH 0, expresses both the greatest concentration of $[H^+]$ ions and the least concentration of $[OH^-]$ ions. The other extreme, pH 14, expresses the reverse situation as regards the concentration of the two kinds of ions. Both kinds are always present throughout the entire range of the pH scale. Moreover ionization is not a constant, or a fixed property of an acid or a base but varies with certain factors, as with temperature. To complete the analogy, "male" potential and "female" potential may be substituted for $[H^+]$ and $[OH]^-$, respectively, on this scale, thus making a correlated gradient of imbalance.

One may ask at this juncture, "What about sex chromosomes?" In the foregoing concept, sex does not reside in sex chromosomes or in their constitutive genes by themselves, nor in the sex chromosomes plus autosomes, nor even in the two gametic nuclei by themselves, but sexual functioning requires the interaction of all of the living protoplasm of the two complementary gametes.

It is of more than passing interest, and very pertinent to the general problems presently under consideration, to point out that sexuality may occur in other Classes of fungi than the three herein discussed. It probably occurs in certain bacteria and slime molds, regarded by some mycologists as fungi, and also in certain Deuteromycetes. This probability rests upon the following facts. None of these organisms possesses structures which may be said to be "male" structures or "female" structures. Evidence of sexuality among bacteria, as summarized by Braun (1953), rests upon genetic analyses. Wilson (1953) recorded the occurrence of syngamy and meiosis in *Dictyostelium*, one of the Acrasiales, a slime-mold like organism.

Gossop et al. (1940) isolated from colonies of *Aspergillus niger*, an organism having black conidial heads, a strain having cinnamon-colored conidial heads and also a strain having brown conidial heads. As a result of hyphal fusion between the cinnamon-spored strain and a black-spored strain they secured colonies having conidial heads of two colors, i.e. cinnamon-colored conidial chains interspersed among black conidial heads. Furthermore, by use of irradiation, Pontecorvo et al. (1953) induced the production of color differences in heads of *Aspergillus niger*, a species not known to possess a sexual cycle, according to the accepted interpretation of sexuality. By growing together the isolates having color differences, they synthesized "diploid strains". Then by genetic analyses of recombinants from the meiotically produced strains they demonstrated the occurrence of segregation. Their results indicate that hyphal fusion with resultant heterocaryosis among certain imperfect fungi may act as "substitute

sexuality". Hansen (1942) employed hyphal fusion and heterocaryosis to account for variability in *Botrytis cinerea*. Davidson, Dowding, and Buller (1932) noted that hyphal fusions occur between strains of the same species of dermatophytes. As a consequence they were able to identify unknown non-sporing strains by mating with known spore-bearing strains.

Structural responses, occurring coincidentally with sex differentiation, which manifest themselves by development of gametangia (spermogonia and oogonia) or other sex organs or structures may then be an outcome or consequence of gametic differentiation. In fact, as Hyndman (1952) states: "Structure and form (in the case of fungi, structure and form of sperms, of eggs, of spermogonia, of oogonia, and of the individuals which bear these structures) are objective consequences, and are developed as material agencies by which function is achieved." Evidence from a series of investigations by Raper (1940, 1950) suggests that all the chemical interaction among fungi as related to sexual structures and processes may one day be found to be hormonally initiated.

If sexuality may be interpreted to be an innate, ever-present property of protoplasm and sex as an entity with two complementary mating potentials, then many of the problems involving differences in patterns of sexual behavior of gametes as related to homothallism and heterothallism appear to be minimized and to a degree to be resolved. As a consequence the seemingly hopeless confusion in the field of sexuality among fungi appears to be supplanted by the realization that there exists a basic unity and harmony among all species.

Lastly the foregoing interpretation of the basic nature of sexuality among fungi raises the intriguing plausibility that this concept may have far-reaching biological significance among other groups of plants and among animals.

SUMMARY

A survey of literature on sexuality of the fungi reveals the existence of apparently widely different morphologic and behavioristic patterns among the different groups. Moreover, there are conflicting opinions among mycologists as to the significance of these differences.

The present account is an effort to clarify and to harmonize these differences, using a concept not previously applied to the sexual behavior of organisms. This concept stems from observations by numerous investigators involving representative species from three of the principal Classes of fungi.

Mycologists regard fungi as either homothallic or heterothallic. If the thallus arising from a single haploid spore produces elements which conjugate, the species is homothallic and, if the interaction of the two different haploid spores or their thalli is required to produce the fertilized cells, the species is heterothallic. One of these two kinds of spores is regarded as having one sex potential and its complementary one as having the other sex potential.

In the present account attention is directed to evidence indicating that each so-called "male" or "female" isolate of a given species is hermaphroditic rather than unisexual, as these terms are commonly defined. If each such isolate is

postulated to be hermaphroditic, i.e. if it is (+ −), then sex difference between gametes or their equivalents may be due to a gradient of imbalance between the factor or factors for maleness (−) and those for femaleness (+). Such imbalance may be induced by sex-differentiating hormones, thus resulting in sex-determination. It follows then that if imbalance is in the (−) direction the predominant mating potential of the gamete is that of a male, and if predominantly in the (+) direction, that of a female.

Imbalance may be of different magnitudes. If on the one hand imbalance is lacking, the gamete is neutral, and if on the other hand imbalance is of sufficient magnitude between two gametes, then the complementary pair may fuse and the zygote results. In due time reduction-division occurs, but binary totipotency is not lost in the resultant haploid nuclei or haploid cells, nor in their progeny.

Sex-differentiation and also sex-determination are fundamentally functional, and by this token, sex itself may not be regarded as morphologic. For sex in all of its manifestations is a series of interrelated and interdependent steps which together are the sex process. Sex organs and sex structures among fungi may not then be deemed as primary but as secondary features.

It might well be that one could regard sexuality as an innate, ever-present property of living protoplasm and could regard sex not as binary but as an indivisible unit or entity having potential for differentiation in complementary directions. From such an interpretation, sex becomes a series of processes by means of which retracement of the species and of all of the features which characterize it, and which constitute the heritable potential, is made possible. From this point of view the "sex-mechanism" or series of co-ordinated processes merely operates to provide the physio-chemical basis for inheritance.

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THE BIOLOGICAL TENET "ALTERNATION OF GENERATIONS"

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Essentially a hundred years ago Wilhelm Hofmeister (1851), Professor of Botany, first at Heidelberg and later at Tübingen, published his classical comparative studies of the development of mosses, ferns, and gymnosperms. Soon thereafter botanists came to appreciate that upon these investigations had been founded a tenet or principle, "Alternation of Generations". According to this concept, plant groups are related evolutionarily to each other through the alternate occurrence of a non-sexual generation or plant, termed sporophyte, bearing spores, and a sexual generation of plant, termed gametophyte, bearing gametes. For a long time now such alternation of the sporophyte generation and gametophyte generation among plants has been widely accepted as axiomatic. Moreover, this tenet has been incorporated as a basic one in text-books for introductory courses, a fact extensively reviewed by Wahl (1945), and has come to be regarded generally as a Gordian knot in correlating structure and development among plants and as indicating phylogenetic relationships among the larger plant groups.

These researches of Hofmeister have stimulated many hundreds of investigations involving the development of sporophyte and gametophyte and repeatedly they have been accorded unstinting praise. Such acclaim is typified by that of Julius Sachs in his History of Botany (1875, p. 215), in which his evaluation is stated as follows: "The conclusion yielded by these 'Comparative Researches' possessed a grandeur unshared by any other work in the domain of descriptive botany. . . . The inner relationships of organisms so extraordinarily diverse as the Liverworts, true Mosses, Ferns, Equiseta, Rhizocarps, Selaginellas, Conifers, Monocotyledons, and Dicotyledons was presented to view with a transparency of relations whereof not the remotest idea was afforded by Systematic Botany down to that date. . . . When, eight years after Hofmeister's 'Comparative Researches' Darwin's doctrine of descent came to light, the relationship in affinity of the great divisions of the vegetable kingdom lay before the eyes, so patent, so deeply founded, and so transparently clear, that the theory of descent only had to recognize what genetic morphology has—as a matter of fact—here brought to view."

It is, at least, quite unusual, perhaps even very presumptuous, for anyone to question the correctness or adequacy of a tenet so generally deemed highly meritorious, and so widely accepted for so long a time. Nevertheless, it is presently intended to reinterpret the facts underlying alternation of generations, so currently understood, and to point out that the principle becomes untenable when viewed in the light of a proper understanding of sexual phenomena among plants. For brevity mainly, the writer has refrained from any consideration of apomixis, apogamy, parthenocarpy, adventive embryony, and polyembryony, in the bearing that these phenomena may have on alternation of generations.

CLARIFICATION OF TERMS

Apparently the phrase, alternation of generations, was first introduced in 1845, by Steenstrup, a Danish investigator (see Harvey-Gibson, 1919, p. 141). About 20 years after the publication of Hofmeister's "Comparative Researches", Celakavosky (1874) made the observation that asexual generations repeatedly might succeed each other among algae and fungi until a sexual generation was produced. But in all other groups of plants, just as Hofmeister had shown, he concluded that the sexual and asexual generation alternately succeed each other with perfect regularity.

Any discussion of the appropriateness or inappropriateness of the terms sporophyte and gametophyte, based on their derivation, is beside the present purpose. It is intended to be interpretative but not to be semantic or controversial. At the outset, however, it is deemed essential to have in mind clearly the accepted usage of these terms and to understand relevant facts. The words, sporophyte and gametophyte, were employed originally to designate morphologic entities or structures. Within the conventionally-accepted life cycle of any given species there was found to be an entity or structure (sporophyte) capable of producing spores. Each spore, in turn, potentially was capable of developing into an entity or structure (gametophyte) capable of producing gametes. Pairs of complementary gametes could fuse, and by so doing could complete the life cycle, and the product of fusion could anew initiate the spore-producing entity.

As stated in Hofmeister's (1851) account, "Mosses and ferns offer accordingly one of the most striking examples of a regular alternation of generations that differ widely in their organization. Of these generations the first, proceeding from the germinating spore, develops antheridia (sperm-producing structures) and archegonia (ovum- or egg-cell-producing structures) which vary in point of numbers. In the central cell of the archegonium, and consequent on fertilization by spermatozoids voided from the antheridia, there arises the second generation. This second generation is destined to produce spores, whereof it always forms a far greater number than that of incipient fruits borne by the first generation."

According to conventionally-accepted usage of terms, the sporophyte and gametophyte attain complete nutritional independence of each other within the group in which are included the ferns and their allies. In all other plant groups, however, the two generations remain organically connected. Hence in them the sporophyte generation is nutritionally dependent upon the gametophyte generation or alternatively the reverse dependence exists. Among all members of such groups one and the same plant may therefore consist of the "gametophytic part, stage, or phase" and the "sporophytic part, stage, or phase".

About 50 years after the publication of Hofmeister's "Comparative Researches", the cytologic findings by Strasburger (see Wilson, 1925), and certain other investigators gave additional support to the belief that sporophyte and gametophyte occur alternately. Each of these several investigators, employing different species, showed that the nuclei of the cells comprising the sporophytic tissues are diploid (2n) whereas those of the gametophytic tissues are haploid (n), i.e. that there are two homologous sets of chromosomes per nucleus in cells of

sporophyte tissue, or twice as many chromosomes per cell as in cells of gametophyte tissue. They established that the haploid condition comes into being following meiosis (reduction-division) and that the resulting spores are the initials of the gametophyte generation. Subsequently, in due time, haploid gametes become differentiated within the gametophyte tissue. But when complementary pairs of such gametes fuse to form the zygote (fertilized egg-cell) the diploid condition is again restored. The zygote, being 2n, is thus the initial stage of the sporophyte generation, completing the cyclic alternation of haploidy and diploidy or of gametophyte and sporophyte.

PHYSIOLOGIC INTERPRETATION

Over the years the terms sporophyte and gametophyte have been employed in such a way as to give the definite impression that alternation of generations involves two distinct structural units. The "phyte", generation, or part, having cells with haploid nuclei, has been regarded as one morphologic entity, and the one having cells with diploid nuclei has been regarded as a different entity. But such confusion arises when structure and function are deemed to be equivalent or interchangeable and synonymous. If function is regarded as basic, however, and structure as adjuvant to function, then a different connotation becomes possible. Haploidy and diploidy, if considered from the point of view of their function in the life cycle of a plant could then actually constitute two alternately occurring phases of the sexual process. With each doubling there must necessarily occur an undoubling. Such alternate doubling and undoubling therefore are manifestations of a series of physiologic phenomena by means of which the sexual process is achieved.

According to the writer's (1954) interpretation, sexuality is an inherent, intrinsic ever-present property of living protoplasts, and sex is an indivisible entity which is not morphologic in any basic sense. Moreover, "sex" structures are themselves consequences of sex-differentiation and accordingly are not to be regarded as causes of sex. The sexual phenomenon in its entirety always consists fundamentally of a series of interdependent, sequential stages or processes (Fig. 1, A–E) as follows: (1) Differentiation of gamete-bearing structures (spermogonia or sperm-bearers and oogonia or egg-bearers). (2) Differentiation of two sets of gametes. The members of one set differ primarily from those of the other set in mating potential. Knowledge of the causes of differentiation of gamete-bearing structures and of gametes is meagre. The causes are generally admitted to be inadequately known beyond the fact that such a differentiation is controlled by auxins or hormones, and hence is considered as among "growth" phenomena. (3) Fusion of gametes in complementary pairs. Two steps are involved, the first of which results in the merging of the cytoplasms into one unit (plasmogamy) and the second in the fusion of paired nuclei (karyogamy). In some species, nuclear fusion regularly is delayed for varying periods and the complementary pair of nuclei may conjugately divide repeatedly with eventual fusion of progeny pairs. When karyogamy has occurred, fertilization or diploidization has been completed and the resulting product is a zygote or diploidized cell. (4) Meiotic division. De-

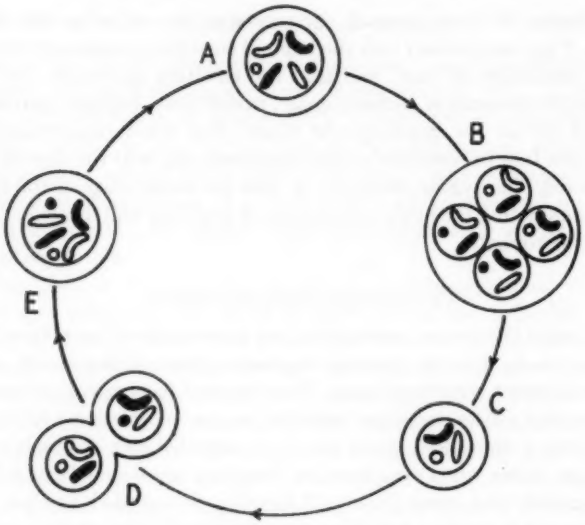

FIG. 1. Diagrams illustrating steps in the sexual process of a plant. A, The zygote or else any cell of tissue developing from the zygote. Each cell-nucleus contains two sets of homologous chromosomes, in this case 3 in each set. The spore-mother-cells of such tissue have a nuclear constitution like A; B, Four haploid spores have arisen by meiotic division of a spore-mother-cell. In this case as a result of segregation each spore is different from each of the others; C, The so-called "gametophyte" or else any haploid cell of the gametophyte. Also C may be any one of the gametes differentiated within a gametophyte; D, A pair of complementary gametes beginning to fuse. Such a pair might be two cells like C, each one of which has n chromosomes; E, Zygote, formed by fusion of two gametes, as in D. Plasmogamy and karyogamy have been completed. Such a zygote is the initial of the so-called "sporophyte".

pending upon the species, meiotic division may occur promptly after karyogamy or else the zygote may first undergo a period of maturation or dormancy. In still other species, a body of tissues, the so-called sporophyte, with cells having diploid nuclei, develops from the zygote. Eventually, sporangia containing spore-mother-cells become differentiated within this body of tissues. Meiosis in such cells involves the nuclei of specialized cells, spore-mother-cells, and as a final result four haploid spores are normally produced from each spore-mother-cell.

These sequential changes, the sexual process, are thus seen to involve both the "sporophyte" and the "gametophyte". If plasmogamy and karyogamy occurred simultaneously or even in rapid succession, and if meiosis followed immediately or soon thereafter in all species of plants, as is well-known to occur in certain species, then there would never have been recognized any part to be termed sporophyte. But, as a matter of fact, the zygote, in such cases, is the so-called sporophyte in its entirety. If all of the stages comprising the sexual process were "telescoped" with concomitant telescoping of the structures commonly regarded

as sporophyte and gametophyte, it becomes easy to comprehend that the two "phytes" should be considered to constitute an entity by means of which the sexual process is carried out. Some botanists, indeed, have so regarded them (Wahl, 1945). Certainly, whether the sequential stages of the sexual process are telescoped, or whether timed lapses intervene, is not in any way of fundamental significance. By this token both sporophyte and gametophyte are structural parts or moieties of one entity or of one generation, the plant. Whether the parts are organically connected is not fundamental. Each part is a functional moiety and the protoplasts of each share the property, sexuality. Reduction in chromosome number at meiosis does not result in loss of sexuality. Neither does any other process in the sequential series comprising the sexual phenomenon. Indeed, sexuality is never lost or acquired *de novo*. The entire interdependent series of sexual processes are never completed by activity of the so-called gametophyte alone nor by the sporophyte alone. But the two structural moieties are complementary, each providing for carrying out steps or stages of the sexual phenomenon and together they constitute a single generation. Neither structure thus can ever lack sexuality, hence neither is asexual or non-sexual.

The present point of view, although approached differently, supports that of Wahl (1945) in maintaining that the sporophyte is not asexual and that it should be considered as part of the sexual life cycle. He states that it is paradoxical to speak of sexual processes, i.e. meiosis, manifested by an asexual generation, but that the paradox is inherent in the terminology, not in the facts. Why textbook writers and teachers of botany should continue to perpetuate the concept of an alternation of sexual and asexual generations, and why they are unwilling to present the facts, is difficult to comprehend, as Wahl too indicates.

Since sexual reproduction so largely conditions the continued existence of plants, it remains a *sine qua non* in the life of the individual organism. Sex thus expresses itself as a sequence of phenomena by means of which inheritance takes place and makes possible dissimilarity between parent and offspring. As for the species, the greater such potential variability among offspring, the greater are its chances of adapting itself to different environments and in this way of assuring the survival of the race.

SUMMARY

The classical tenet, alternation of generations, is held to be untenable, because sex is functional, not structural. The terms sporophyte and gametophyte connote structural moieties of an entity, a single generation, which is the plant. Conceivably, a useful purpose might be served if the two terms were retained with their meaning restricted to structure alone. But structure and function cannot be employed as interchangeable concepts. If, instead of interpreting sexuality among plants as fundamentally morphologic, it is deemed to be a series of sequential phenomena, then the changes incident to haploidy and diploidy are merely stages in the sexual process. And if function of sporophyte and gametophyte is emphasized then the two structures are appreciated to be complementary in making possible the steps in the sexual process and neither of them is non-sexual.

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WHAT ARE FUNGI?

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For a number of years the writer has been concerned, perhaps unduly, with an appropriate answer to the question "What are fungi?" Mycologists generally, if asked this question, respond that an entirely satisfactory answer already exists and that no purpose is served in asking so pointless and trite a question, one about which almost complete unanimity of opinion has been established for more than 75 years. Admittedly this opinion has ponderous support. However, none, including the present writer, will contend that he is able adequately to delimit or otherwise to describe or define fungi by use of a few sentences. But to students being introduced to a study of fungi a consideration of this question may have merit, especially if it were indicated that accepted concepts of the nature of fungi do not provide for a proper understanding of their metabolic activities, and hence of their place in the economy of nature. If this aspect of fungi were to be brought into perspective when a study of mycology is first begun it is conceivable that such a point of view would have a tremendous impact upon the emphasis in future studies of this group of organisms.

It is intended herein to reconsider the query by stressing that the field involving the metabolic potential of fungi has been under-emphasized and has too long been unappreciated and that it might be explored further with profit. The need for such explorations is indicated by results obtained during recent years which focus attention on the fact that fungi possess not only important analytic capabilities but also numerous synthetic capabilities.

CURRENT CONCEPTS

Realization of the difficulties inherent in stating briefly what fungi are and what they may do may be appreciated best by consulting statements contained in

recent mycological books, as those by Bessey (1950), Gäumann and Wynd (1952) and Alexopoulos (1952). These brief statements are the currently and generally accepted concepts of the nature of fungi.

Bessey (1950) states: "Fungi may be defined as chlorophyll-less nonvascular plants whose vegetative and reproductive structures do not permit them to be assigned to positions among recognized groups of algae or higher plants, and as excluding Bacteria and the Mycetozoa. . . . Because of their lack of chlorophyll all fungi must obtain their organic food from sources external to themselves. . . . Probably the majority of fungi are saprophytic, i.e., feed upon the organic products or remains of plants or animals, but not upon the living organisms themselves. Many fungi are parasitic in certain stages of their growth and saprophytic later on".

The statements by Gäumann and Wynd (1952) are quite in accord with those by Bessey. They point out: "The fungi are those thallophytes which do not possess chlorophyll. The Bacteria and Myxomycetes, although they also are thallophytes without chlorophyll, usually are excluded from the fungi because of certain features of their structure and life history. . . . The evolutionary development of the fungi resulted from disturbances of the nutritional physiology of their algal ancestors. Their evolutionary descent from the green algae was accompanied by the loss of chlorophyll which led them to a parasitic or saprophytic mode of life. Many types also lost their ability to synthesize growth hormones and other physiologically important substances when their photosynthetic processes were interrupted by the loss of chlorophyll."

In delimiting fungi, Alexopoulos (1952) states "since we now believe that the bacteria and probably the slime molds have had a different origin from the true fungi, we restrict, at present, the term fungus to the nucleated, achlorophyllous organisms which typically reproduce sexually and asexually, and whose usually filamentous, branched, somatic structures are surrounded by cell wall. . . . Fungi obtain their food either by infecting living organisms as parasites or by attacking dead organic matters as saprobes. . . . Fungi differ from most plants in that they require already elaborated food in order to live, and are incapable of manufacturing their own. But if given carbohydrates in some form—preferably glucose, sucrose or maltose—most fungi can synthesize their own proteins by utilizing inorganic or organic sources of nitrogen. Many fungi are capable of synthesizing vitamins which they require for growth and reproduction as do other organisms. Fungi usually store excess food in the form of glycogen or oil."

It is apparent that the foregoing statements of the characteristics of fungi fundamentally do not differ and that they are in essence evolutionary and phylogenetic. In each, the fungi are regarded as most probably of algal ancestry, although the likelihood of protozoan or other origin is not excluded. In a final chapter in the volume by Bessey the algal origin, in fact, is elaborated in detail, using diagrams of probable phylogeny, as based upon morphology. Each foregoing concept contains the stated or implied viewpoint that degradation has occurred and that such degradation is a consequence of loss of chlorophyll and hence of loss of photosynethetic ability. Furthermore, each characterization implies that the nutritional requirements of any given fungus are met by utilization of what-

ever materials exist within the specific substratum, i.e. within either living or non-living tissues of animals or plants.

The classical basis for these concepts of the nature of fungi undoubtedly rests upon numerous researches in the field of developmental morphology initiated and fostered by De Bary and continued by his students and, in turn, by those whom they instructed. As an outcome, the solution of all problems dealing with taxonomy, classification, evolution, and phylogeny of fungi presently has come to be founded upon the morphological approach. Proximity of relationship or of origin, from this viewpoint, is reflected by structural similarities, those organisms most closely related being most nearly alike anatomically. In fact, biologists have come to accept the correctness of such interpretation as axiomatic. Whether morphologic similarities constitute the sole basis for determining relationships among fungi, however, may be brought into question. Certainly one should not preclude the possibility that other bases such as their nutritional and biochemical potentials may have significance in indicating relationships.

As has been implied, a few mycologists have expressed a different viewpoint on the nature of fungi. Among such is Dodge (1939) who, in summarizing his ideas, prophetically stated that "the fungi are not degenerate organisms on their way out in the scheme of evolution, and so of little economic importance and scientific interest. The fungi, on the contrary, are progressive, ever changing and evolving rapidly in their own way so that they are becoming rapidly adapted to every condition of life. We may rest assured that as green plants and animals disappear one by one from the face of the globe, some fungi willl aways be present to dispose of the last remains."

METABOLIC ACTIVITIES OF FUNGI

Undoubtedly few if any mycologists would now contend that the words parasitic and saprophytic, as defined in standard dictionaries, adequately delineate the metabolic capabilities of any given fungus. For it becomes apparent to anyone who cultivates a given species on artificial media that any so-called parasite or so-called saprophyte may not be limited in catabolic activities to a single substratum. Instead, the given species may be able to catabolize a wide variety of nutrients and also may be able, at the same time, to anabolize a wide variety of products. As a consequence, one is made to realize that both parasites and saprophytes possess both an analytic and a synthetic potential, thus bringing into question the continued usefulness of the terms parasite and saprophyte. Even though mycologists are well aware of these basic features of fungus nutrition they appear to regard them as minor and instead center attention on matters pertaining to morphology, reproduction, taxonomy and nomenclature. Such matters certainly remain very essential in the study of fungi but at the same time one may regard them as inadequate in providing a comprehensive understanding of the given fungus.

No definite date can be assigned to mark the beginning of appreciation that biochemical transformations may be accomplished by micro-organisms. Certain pertinent knowledge of such occurrence was employed even in ancient times. The

impetus for present-day investigations, however, may be assumed to have been provided by the researches of Raistrick, begun about 25 years ago. He early came to regard fungi as polychemic agencies. An introduction to the plethora of findings on their polychemism is available in a volume by Foster (1949).

One of the most important factors in studying the biochemical potential of fungi has been the development and use of chemically defined media. Such media contain the mineral elements known to occur normally in plants, plus organic or inorganic carbon sources and/or nitrogen sources, added singly. Such substrata supply the various fungi with materials from which they get energy and from which they elaborate many organic compounds, some of which they employ as food, some of which they store as reserve, and some of which aid in respiration and the many other metabolic processes involved in growth and reproduction. If one turns his interest to finding out what sorts of products fungi may synthesize, as compared with those elaborated by green plants, he will perceive that the two groups of organisms have much in common. Among carbon compounds it will be found that fungi synthesize sugars, starches, celluloses, glycogen, chitin and many kinds of organic acids including such familiar ones as oxalic, acetic, citric, malic, fumaric, succinic, tartaric, etc. of common occurrence in green plant tissues. Besides, many other organic acids which green plants do not seem to be able to synthesize will be found to be elaborated by specific fungi. Among nitrogen compounds, most fungi will be found capable of elaborating their own amino acids, some being able to use inorganic sources of nitrogen, from which they build their proteins. As regards fat metabolism, they elaborate glycerol and various fatty acids to provide the food reserve of oil so commonly present in hyphal cells and spores. Besides these compounds one must add to the list of metabolic products which fungi synthesize such other compounds as vitamins and growth accessory or regulatory substances, pigments, toxins, sterols, antibiotics, etc. A comprehensive list of products anabolized by fungi has never been prepared. Certainly their presently known number would exceed several hundred and there are reasons for believing that such a list, if prepared 25 years hence, would include not a few hundred but several thousand different products.

DISCUSSION

Admittedly none of the foregoing facts learned from this sketchy survey of the biochemical potential of fungi is novel to mycologists. The facts showing their polychemic capabilities previously may have been marshalled better, but this does not detract from the conclusion that fungi, in spite of lack of chlorophyll, are not at any real disadvantage when their synthesizing potential is compared with that of green plants. Therefore it becomes doubtful whether a penalty has been assessed against fungi because of lack of chlorophyll and consequently whether they should be regarded as degraded.

Certainly in the sense that fungi lack roots, stems, and leaves, they are thallophytes. But this connotation need not imply that there is phyletic relationship between fungi and algae. At present there seems little likelihood that accord on

the ancestry of fungi can be reached. Moreover, accord does not exist as to the origin of the Classes and constitutive Orders of fungi. The phyletic lines are obviously much branched, devious, and not well marked. Certain branches appear severed or detached and some appear to be widely separated spatially from the others. This opinion is based upon the heterogeneity of structure encountered among fungi which is of a magnitude and complexity probably not exceeded by any other group of organisms.

Finally, it is the writer's opinion that so long as mycologic research and the teaching of mycology are so largely based upon the classical concept which emphasizes morphology, reproduction, parasitism, and saprophytism, the nature of fungi will remain misunderstood, and bacteria and slime molds will remain excluded from fungi. Moreover, so long as this approach prevails, little real progress can be expected in knowledge relating to the nutrition, physiology, ecology, and biochemical activities of fungi. Also interrelationships between bacteria, slime molds, and "true" fungi will remain problematic and accord will be lacking on how the term fungi should be characterized or delimited.

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AN ANALYSIS OF VARIATION WITHIN MAGNOLIA ACUMINATA L.

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INTRODUCTION

The Cucumber-tree, *Magnolia acuminata* L., is a well known plant of cultivation and as a constituent of deciduous forests in eastern North America. The variation within this species has been a source of disturbance to many botanists, particularly those in the southeastern United States where many of the extremes of variation occur.

[1] The author wishes to express his appreciation to Professor Rogers McVaugh, University of Michigan, for his helpful suggestions offered during the progress of this study and for his constructive criticism in the preparation of the manuscript. The aid of Dora Mondon, who made the statistical calculations, is also gratefully acknowledged.

Magnolia acuminata has been described under two generic, two specific and six varietal names involving some sixteen or more different combinations, all published prior to 1932. Those who described the varieties worked for the most part on a restricted number of specimens representing only a relatively small segment of the entire geographic range of the species. Resulting from this have been various opinions as to the validity of the described species and varieties. In the recent check list by Little (1953) only one variety beside the typical is recognized as being distinct.

The purpose of the present study has been to analyze the pattern of variation as shown by herbarium specimens representing the entire range of distribution. Two questions arise. Does the pattern of variation show infraspecific categories and if so what is the rank of each? The derived conclusions are presented here.

METHODS OF ANALYSIS AND PRESENT CONCEPTS

Specimens from eight herbaria[2] were studied and the following data recorded as completely as possible.

State, County, Collector and Number, Date
Life form and habitat, as recorded by collector
Leaf: position, shape of base and apex, point of maximum width, pubescence of upper and lower surfaces, petiole length and pubescence, color of lower surface
Twig: pubescence of nodes and internodes
Flower: petal length and width, color (as recorded by collector), stamen length, pubescence of peduncle.

Such tabulated data are easily handled for making various correlations and statistical analyses, some of which are discussed later. A few of the characters recorded were found to be insignificant, varying either slightly, or widely but without pattern. Leaf apex varied tremendously, but its variation could not be correlated with other morphological characters or with geographic range. The point of maximum leaf width, pubescence of upper surface, color of leaf, stamen length, life form and habitat varied only slightly and without significance. Ecological data had been rarely recorded by the collectors, making it impossible to determine the significance of this factor which could prove to be so important in areas where varieties overlap in range.

In addition to gross morphological features, the leaf anatomy was briefly examined to determine the importance of such a character in the over-all picture of infraspecific variation. For each of the described varieties, one leaf was removed from a specimen which had many overlapping leaves. Similar portions of these leaves were cleared, stained and mounted. The sizes and shapes of vein islets were essentially alike, lobing of epidermal cells was of a similar pattern and all trichomes were of a linear 1–3-celled type with thin walls. Thus there were no significant variations in leaf anatomy within the species as shown by these methods.

[2] Arnold Arboretum (74) specimens), Univ. of Georgia (79), Univ. of Michigan (11), Missouri Bot. Gard. (89), U. S. National Herb. (91), New York Bot. Gard. (72), Univ. of North Carolina (135) and Univ. of Tennessee (36). The author is indebted to the curators for the loan of these specimens.

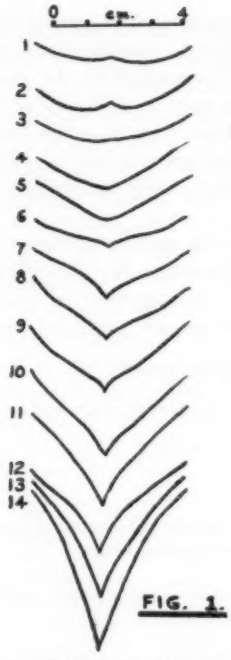


FIG. 1.

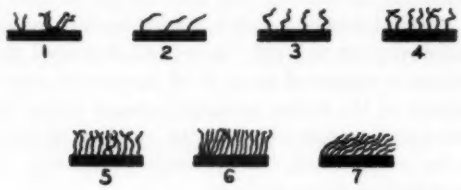


FIG. 2.

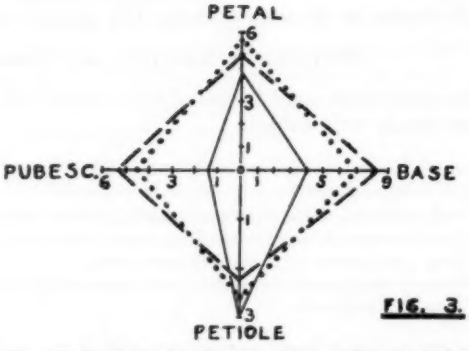


FIG. 3.

FIG. 1. Scoring of leaf bases. FIG. 2. Scoring of pubescent types. FIG. 3. Polygonal graph of character means. Solid line, *ozarkensis*; dashes, *cordata*; dots, *acuminata*.

Certain characters immediately showed a definite pattern of variation which was easily correlated with other morphological characters and with geographical range. These characters, which remain as most significant in distinguishing the infraspecific categories, are: leaf base, pubescence of lower leaf surface, twig pubescence, petal length and width, flower color and pubescence of peduncle.

At the outset of this study it was thought that leaf shape varied according to position on the branch and twig.[3] The position was recorded, therefore, for each leaf analyzed. The first-formed or lowest leaf on a fertile twig was designated as position I and used when possible. The alternative position (II) was the subterminal leaf on a sterile twig. The actual differences are shown later under the statistical analysis.

Leaf shape in *M. acuminata* is by far the most variable of the morphological characters studied. The precise methods of measurement used by Woodson (1947) for *Asclepias* could not be adopted for this species. The technique finally used to record base shapes involved a series of outlines (Fig. 1), originally traced from leaves onto transparent paper, which were arranged in sequence from subcordate to acuminate and assigned numerical values from 1 to 14. Each specimen was then matched with an outline and its corresponding number recorded.

[3] Throughout this paper the use of "twig" refers only to the current season's growth, terminated basally by the circumferential terminal-bud scar.

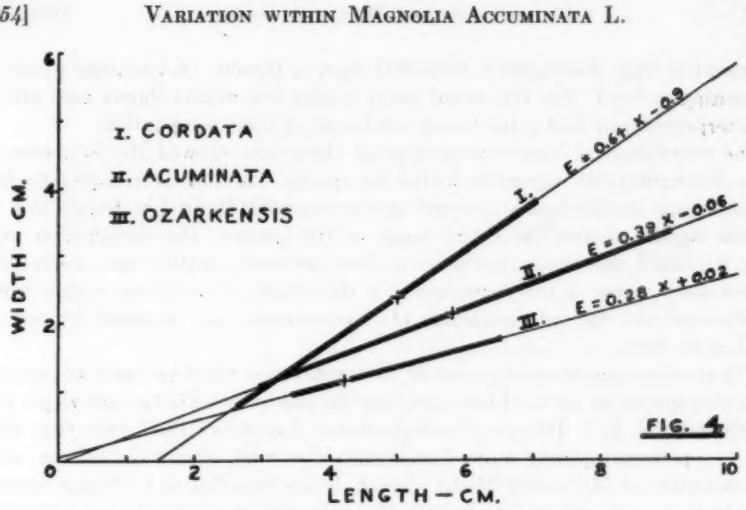


FIG. 4. Regression of petal width on length.

Apices were similarly scored but are not illustrated here since they were not used in the final analysis. The shapes of the apices, however, varied from round to acuminate and apiculate.

The degrees of pubescence found on the lower leaf surface were numerically scored from 1 to 7 corresponding to a series from glabrous or extremely sparse (1) through tomentose to sericeous (7). The types of pubescence illustrated (Fig. 2) were drawn during a preliminary survey of the leaves and later used in matching with each specimen. The illustrations do not show length of hairs exactly to scale, but rather give the aspect of relative abundance over equal areas.

As a characteristic of all the varieties of the species, the twigs are slightly pubescent or often villous at the terminal-bud scar at the base of the twig and on the upper and lower internodes immediately adjacent to this scar. For varietal distinction, therefore, the significant locations for pubescence are the remaining internodes of the current and previous year's growth. Often by the end of the first year the pubescence of these positions is lost; however, if originally present, the bases of the trichomes very often remain and may be seen with magnification. The presence or absence of stem pubescence is one of the more important taxonomic characters within the species. The two extremes were recorded as plus or minus respectively.

Measurements were made of the maximum dimensions of the largest petal, and color recorded when on the label. Indication of color, which is an important character within the species, was sadly lacking from the labels and the original color was rarely preserved. The peduncle varies from a completely glabrous contition to extremely villous. This character was also recorded as plus or minus indicating presence or absence of pubescence.

An analysis of herbarium specimens alone is inadequate for revealing the true relationships and variations within the species. The sheets examined, however,

represent a wide geographical area and show a pattern of variation which is interesting in itself. The important point is that the results shown here are to be interpreted only under the broad conditions of their compilation.

The correlation of these morphological characters showed the existence of three distinguishable segregates within the species. Each of these segregates has characteristic morphological features and is somewhat limited in distribution to certain regions within the entire range of the species. The distribution map (Fig. 6), based only upon specimens studied, presents a rather false impression of the distinctness of the segregates, for the details of variation within small populations and the intergradation of characteristics are obscured by such a small-scale map.

The general opinion among some of the taxonomists who have been acquainted with this species in the field has been that the described varieties are vague and poorly defined. E. J. Palmer (Correspondence, November 1953) feels that they are "... perhaps nothing more than forms, if worthy of any distinction, with the exception of *M. cordata* Michx., which, if not regarded as a distinct species, is at least a good variety." Coker (1943), although presenting a seemingly clear distinction among three varieties, stated: "We are evidently dealing with a very variable group with extremes connected by a series of forms." He went on to say that "... we arrive at the disappointing conclusion that *cordata* is only a rather vaguely defined, marginal extension of the southern yellow-flowered variety of *M. acuminata*."

Because of the intergradation of characters and great variability in populations where these segregates overlap in distribution, they are considered as only varieties of the species. A *variety* is considered here as a morphological variant having a more or less definite geographical distribution, but often sharing this area with another variety of the same species. Tryon (1941) brings out three interesting points concerning varieties. He states that intermediate forms are found where varieties overlap, that some individuals may resemble another variety entirely except for the "geographical character", and that key characters for two varieties may be quite insignificant for purposes of identifying other varieties.

Stem of the current and previous year glabrous, with tomentum only at the terminal-bud scar and on the upper and lower adjacent internode to this scar; petals greenish to greenish-yellow throughout, or greenish-yellow outside and golden yellow inside, 3–9 cm. long; peduncle glabrous or rarely villous; leaf tomentose to glabrous below.

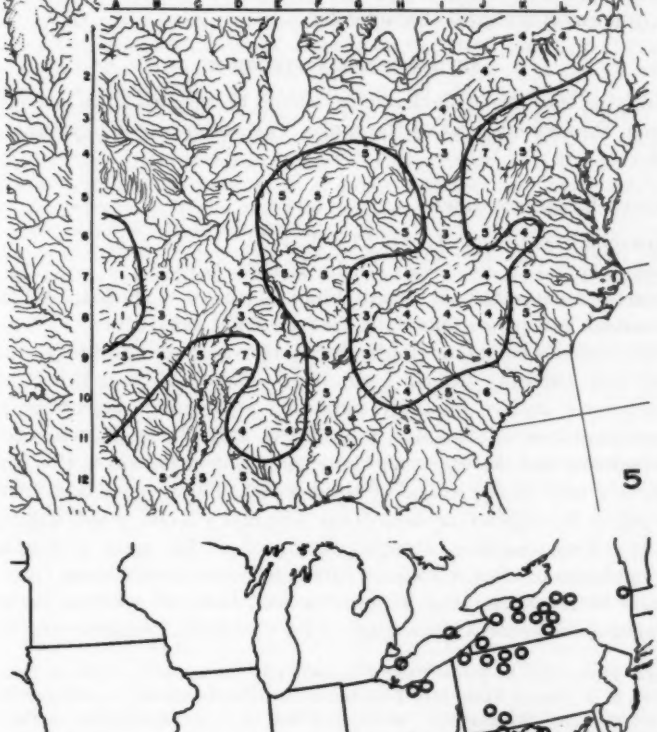
Leaves tomentose on lower surface; base rounded, truncate, cuneate, acuminate or rarely subcordate.

Flowers greenish to greenish-yellow throughout ..... var. *acuminata*.

Flowers greenish-yellow on outside and clear golden yellow on inner petals ..... forma *aurea*.

Leaves glabrous or early glabrate with scattered hairs remaining on veins of lower surface; base mostly rounded, truncate or subcordate, rarely cuneate or acute ..... var. *ozarkensis*.

Stem of current and previous year with short tomentum or roughened with bases of hairs; petals greenish-yellow throughout or more often light yellow outside and golden yellow inside, 2.5–7 cm. long; peduncle typically villous, rarely glabrous; leaf tomentose below ..... var. *cordata*.



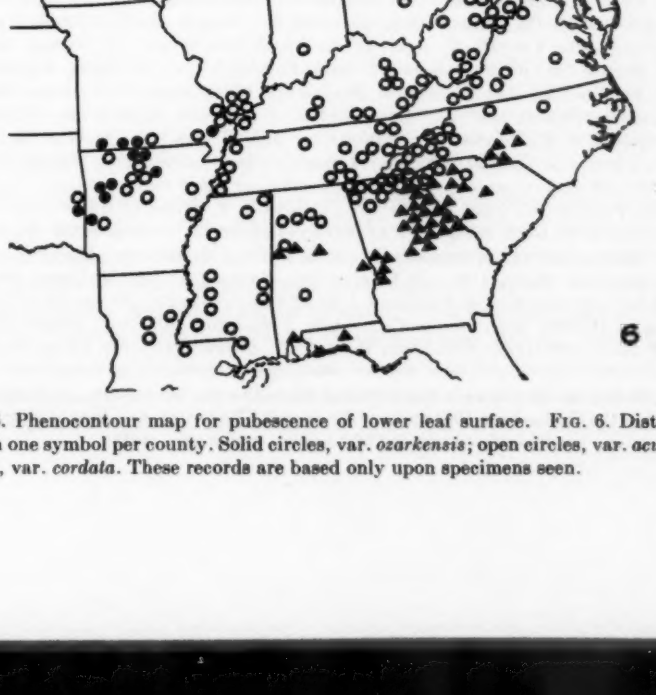


FIG. 5. Phenocontour map for pubescence of lower leaf surface. FIG. 6. Distribution map with one symbol per county. Solid circles, var. *ozarkensis*; open circles, var. *acuminata*; triangles, var. *cordata*. These records are based only upon specimens seen.

A *forma* or form of a variety constitutes merely a trivial variation such as flower color occurring among individuals of any population and not correlated with any other morphological characteristic.

INFRASPECIFIC CATEGORIES

In identifying specimens, all characters should be weighed since intermediates are common throughout most of the range. The accompanying synoptic key is presented.

M. acuminata L. var. *acuminata*

M. virginiana e *acuminata* L., Sp. Pl. 536. 1753.
M. acuminata (L.) L., Syst. Nat. Ed. 10, **2:** 1082. 1759.
Tulipastrum acuminatum (L.) Small, Flora Southeast. U. S. 451, 1331. 1903.
M. acuminata var. *ludoviciana* Sarg., Bot. Gaz. **67:** 232. 1919.

Leaf thin, broadly oblong or elliptic, rarely obovate; apex abruptly acuminate to obtuse; base typically truncate, but often subcordate, rounded, cuneate or acuminate; lower surface minutely pubescent to densely tomentose; petioles slender, pubescent on adaxial side, 1.5–4.5 cm. long. Twig glabrous except for terminal-bud scar and the upper and lower adjacent internode to this scar; red-brown turning gray in the second or third season. Flower glaucous, greenish to greenish-yellow throughout; petals 3–9 cm. long and 1–3 cm. broad, oblanceloate, cuneate or oblong-spatulate, abruptly narrowed at the apex; peduncle 1.5–3 cm. long, glabrous or often villous.—Distributed over a wide range from southern Ontario to Louisiana (Fig. 6, open circles), however reaching its greatest size and abundance in the Appalachians of the Carolinas, Tennessee and Georgia.

ALABAMA: Blount Co.,[4] *Charles Mohr*, 6/1/1882 (US); Cullman Co., *Mohr*, 6/1/1882 (US); Marion Co., *E. J. Palmer 38992* (A); Tuscaloosa Co., *Palmer 35377* (A); Wilcox Co., *L. B. Buckley*, 4/1840 (MO); Winston Co., *Mohr*, 5/2/1881 (A). ARKANSAS: Carroll Co., *Palmer 20511* (A); Cross Co., *Palmer 31659* (A); Faulkner Co., *Palmer 26540* (A); Marion Co., *Palmer 5926* (MICH); Phillips Co., *Palmer 25104* (A); Polk Co., *Palmer 23965* (A); Pope Co., *G. M. Merrill 5* (A); Stone Co., *W. W. Ashe*, 5/7/24 (NCU); Van Buren Co., *Palmer 24307* (A). GEORGIA: Bartow Co., *W. H. Duncan 8171* (GA); Catoosa Co., *J. K. Small*, 8/6/1895 (NY); Dade Co., *Duncan 2404* (GA); Fannin Co., *Duncan 5494* (GA); Gilmer Co., *Duncan 2821* (GA); Habersham Co., *Duncan 1554* (GA); Lumpkin Co., *J. H. Miller*, 6/29/40 (GA); Murray Co., *Duncan 16454 & J. W. Hardin* (GA); Rabun Co., *Duncan 7518* (GA); Stevens Co., *Duncan 9857* (GA); Towns Co., *Duncan 6496a* (GA); Union Co., *Duncan 2905* (GA); Walker Co., *H. W. Harvey*, 5/19/40 (GA). ILLINOIS: Alexander Co., *Palmer 14903* (MO); Johnson Co., *Palmer 16673* (NY); Pulaski Co., *Palmer 14869* (MO); Union Co., *L. Hubricht B2268* (NY). INDIANA: Lawrence Co., *C. C. Deam 26184* (NY). KENTUCKY: Ballard Co., *Palmer 15109* (A); Breathitt Co., *R. E. Horsey 1119* (A); Edmonson Co., *Sadie F. Price*, 7/1898 (MO); Harlan Co., *Horsey 1987* (A); Lee Co., *Horsey 1157* (A); Letcher Co., *Horsey 1217* (A); McCreary Co., *H. J. Rogers 13* (NY); Menifee Co., *McFarland & Lyle 4272* (MO); Pike Co., *Horsey 1200* (A); Warren Co., *Price*, 5/15/1899 (MO); Wayne Co., *Smith & Hodgdon 3867* (NY); Wolfe Co., *Price*, 6/15/1898 (MO). LOUISIANA: Catahoula Par., *C. A. Brown 7394* (GA); Grant Par., *Ashe*,

[4] The citation of specimens is selective and limited to one per county. A complete list is deposited in the Herbarium, University of Michigan. The letters used here as abbreviations for the herbaria are those recommended by Lanjouw and Stafleu (1952).

3/29/27 (NCU); Rapides Par., *Caroline Dormon*, 6/13/29 (NCU); West Feliciana Par., *C. S. Sargent*, 4/12/16 (A). MARYLAND: Garrett Co., *J. D. Smith*, 7/6/1878 (US). MISSISSIPPI: Clarke Co., *Mohr*, 6/23/1895 (A); Copriah Co., *Ashe*, 6/13/29 (NCU); Franklin Co., *T. G. Harbison*, 7/8/30 (NCU); Hinds Co., *Harbison 5* (A); Lauderdale Co., *W. B. Allison*, 4/18/47 (A); Marion Co., *Ashe*, 5/10/30 (NCU); Pearl River Co., *Ashe*, 5/8/13 (NCU); Tallahatchie Co., *Duncan 15417 & Hardin* (GA); Tishomingo Co., *Harbison 25* (A); Union Co., *Harbison 19* (A); Yazoo Co., *Harbison 11919* (NCU). MISSOURI: Butler Co., *Palmer 16394* (MO); Cape Girardeau Co., *Palmer 18003* (MO); Ozark Co., *Palmer 33199* (MO); Scott Co., *Palmer 34892* (MO). NEW YORK: Chautauqua Co., *J. R. Churchill*, 8/5/1896 (MO); Chemung Co., *T. F. Lucy 242* (US); Erie Co., *D. F. Day*, 6/1904 (NY); Livingston Co., *F. W. Johnson*, 5/29/27 (US); Madison Co., *H. D. House 25321* (NY); Monroe Co., *M. S. Baxter*, 6/20/1877 (NY); Onondaga Co., *Cowles 204* (MO); Rensselaer Co., *R. McVaugh 3786* (GA); Seneca Co., *J. W. Checkering, Jr.*, 6/1857 (US); Tompkins Co., *W. Manning 16132* (MO); Yates Co., *S. H. Wright* (NY). NORTH CAROLINA: Avery Co., *G. B. Sudworth*, 8/10/1890 (US); Buncomb Co., *Biltmore Herb. 1357b* (MICH); Burke Co., Torrey Herb. (NY); Haywood Co., *N. L. Britton*, 9/18/1895 (NY); Jackson Co., *E. R. Memminger*, 8/1888 (NY); Mitchell Co., *J. H. Redfield 11678* (MO), Polk Co., *H. R. Totten*, 4/29/34 (NCU); Swain Co., *W. B. Fox 772* (GA); Stokes Co., *Fox 840* (NCU); Yancey Co., *A. E. Radford*, 7/9/46 (NCU). OHIO: Cuyahoga Co., *G. B. Ashcraft 242* (MICH); Fairfield Co., *M. L. Fisher*, 9/3/1898 (NY); Lorain Co., *R. M. Strong*, 5/30/1892 (MICH). OKLAHOMA: LeFlore Co., *Palmer 12637* (MO). ONTARIO, Canada: *Macoun, Geol. & Nat. Hist. Survey Canada* 80 (US). PENNSYLVANIA: Elk Co., *Ashe*, 8/8/24 (NCU); Erie Co., *E. A. Southworth*, 6/1868 (MICH); McKean Co., *Ashe*, 8/8/24 (NCU); Potter Co., *A. M. Neefe*, 7/1895 (US); Somerset Co., *H. N. Moldenke 3870* (NY); Warren Co., *Ashe*, 8/6/24 (NCU); Westmoreland Co., *P. E. Pierron*, 5/6/1878 (MO); Wyoming Co., *Ruth Herb. 242* (TENN). SOUTH CAROLINA: Oconee Co., *Ashe*, 6/24/24 (NCU); Pickens Co., *Duncan 13962 & Hardin* (GA); Union Co., *Duncan 15691 & Hardin* (GA). TENNESSEE: Blount Co., *H. M. Jennison 189* (TENN); Campbell Co., *Sharpe, UTenn 3801* (TENN); Davidson Co., *A. Gattinger* (NY); Franklin Co., *Shanks et al., UTenn 5368* (TENN); Grundy Co., *Duncan 6194* (GA); Lauderdale Co., *Sharp et al., UTenn 7948* (TENN); Marion Co., *Sharp et al., UTenn 55-1948* (TENN); Obion Co., *Shanks et al., UTenn 15865* (TENN); Rhea Co., *Shanks et al., UTenn 4318* (TENN); Roane Co., *D. M. Coffman*, 5/2/1891 (US); Shelby Co., *Sharp et al., UTenn 6455* (TENN); Tipton Co., *Sharp et al. UTenn 6403* (TENN); Union Co., *A. W. Morrison*, 6/2/37 (TENN); White Co., *Shanks et al., UTenn 5568* (TENN). VIRGINIA: Bedford Co., *A. H. Curtiss*, 5/15/1874 (MO); Chesterfield Co., *A. B. Massey*, 5/13/45 (NCU); Franklin Co., *Totten*, 8/25/34 (NCU); Giles Co., *W. M. Canby*, 5/1869 (NY); Shenandoah Co., *H. A. Allard 4894* (US); Smyth Co., *Small*, 7/30/1892 (TENN); Wythe Co., *E. C. & G. M. Leonard 16339* (US). WEST VIRGINIA: Fayette Co., *Maxon & Pollard 46* (US); Greenbrier Co., *J. Dotts, N. Y. State College For. 8360* (NY); Hardy Co., *H. Hechenbleikner*, 6/9/39 (NCU); Jefferson Co., *C. L. Pollard*, 5/4/1898 (US); Mercer Co., *Ashe* (NCU); Monongalia Co., *C. F. Millspaugh 125* (NY); Pendleton Co., *Allard 3608* (US); Preston Co., *E. S. Steele*, 8/16/1898 (NY); Randolph Co., *Ashe*, 8/13/24 (NCU); Summers Co., *W. W. Eggleston 5568* (NY); Tucker Co., *Ashe* (NCU); Upshur Co., *W. M. Pollock*, 5/23/1897 (MO); Wirt Co., *Eliz. A. Bartholomew W-1348* (US).

The var. *ludoviciana* was described by Sargent from specimens collected by Cocks and himself in 1914–1915 at two localities in West Feliciana Parish, Louisiana. Among specimens located in the Herbarium of the Arnold Arboretum, were collections by Sargent and marked as type (syntypes). From these, one specimen is hereby designated as the lectotype. This specimen was collected by Sargent on April 12, 1916 from Desert Plantation near Catalpa, West Feliciana Parish, Louisiana and represents the most complete and typical of his collections. The bases for segregation were the extremely large flowers (8–10 cm. long) and heavily tomentose leaves; these characters, however, are not consistent and do

not appear sufficiently distinct from var. *acuminata* to warrant varietal segregation.

M. acuminata forma *aurea* (Ashe) Hardin; ***comb. nov.***

Tulipastrum acuminatum aureum Ashe, Charleston Museum Bull. **13:** 28. 1917.

M. acuminata [var.] *aurea* (Ashe) Ashe, Torreya **31:** 38. 1931.

Outer petals green with yellow tint but slightly more yellow inside and toward the tip; inner petals clear golden yellow (Wax Yellow; Ridgway, 1912) tinted with green only toward the base.—This color form is found scattered in the mountains and upper Piedmont of the Carolinas, Tennessee, Georgia and possibly Alabama.

GEORGIA: Towns Co., *W. H. Duncan 16319 & J. W. Hardin* (GA). NORTH CAROLINA: Haywood Co., *H. D. House 5129* (NY); Macon Co., *H. Wright*, 7/29/42 (NCU); Madison Co., *W. L. Hunt*, 4/22/38 (NCU). SOUTH CAROLINA: Greenville Co., *W. C. Coker*, 5/7/37 (NCU). TENNESSEE: Sevier Co., *A. Stupka 10047* (TENN).

The type specimens upon which Ashe based his var. *aureum* have not been located by the author. From Ashe's original description it seems possible that this variety was described on the basis of his general knowledge of the group and not from any one or more specimens. All collections of the species will have to be checked before one is able to determine the exact situation concerning the type specimen.

This yellow-flowered form in the mountains was included within the concept of *cordata* until 1917. This is shown by the early manuals of Torrey and Gray (1838), Chapman (1860) and Small (1903, 1933), and is understandable on the basis of the original description of *M. cordata* Michx. in 1803 ("M. foliis cordatis, subtus subtomentosis: floribus flavis") which is general enough to include both. After the original population of *cordata* was rediscovered by L. A. Berckmans in 1913, Ashe, in describing var. *aureum*, made the separation between the Appalachian yellow-flowered form and *cordata* of the Piedmont. Coker and Totten (1945) found only the yellow-flowered form in the mountains. More specimens indicate, however, that both var. *acuminata* and its forma *aurea* exist together in the Appalachians.

The flower color is the only character found to separate these yellow-flowered forms with glabrous twigs. It is necessary, therefore, to reduce the original variety to a forma *aurea* of var. *acuminata*. Actually, when Ashe proposed his variety he stated that it was possibly only a form of the typical *acuminata*.

M. acuminata var. *ozarkensis* Ashe, Elisha Mitchell Sci. Soc. Jour. **41:** 269, 1926.

Tulipastrum acuminatum var. *ozarkense* (Ashe) Ashe, Torrey Bot. Club Bull. **55:**464. 1928.

Leaf thin, broadly obovate, oval, ovate or elliptic; apex abruptly short-pointed to actue or acuminate; base typically rounded, but often truncate to rarely acute; lower surface slightly tomentose when young, soon becoming

sparsely pubescent with scattered hairs along the main veins; petioles slender, pubescent on the adaxial surface, 2–5 cm. long. Twig glabrous except for the terminal-bud scar and the upper and lower adjacent internode; red-brown becoming gray in second or third season. Flower greenish-yellow to rarely purplish tinged; petals 4.5–6.5 cm. long and 1–2 cm. broad, oblanceolate or oblong-spatulate; peduncle glabrous.—Distributed over a limited range in the Ouachita and Boston mountains of western Arkansas and eastern Oklahoma, and the Ozark Plateau of southern Missouri (Fig. 6, solid circles).

ARKANSAS: Baxter Co., *D. Demaree 3089* (MO); Franklin Co., *W. W. Ashe 17* (NCU); Johnson Co., *Ashe 7* (Type, NY); Logan Co., *E. J. Palmer 23238* (MO); Marion Co., *Palmer 20663* (A); Polk Co., *Demaree 6200* (TENN); Stone Co., *Ashe*, 6/7/24 (NCU). MISSOURI: Barry Co., *B. F. Bush 4* (NY); Ozark Co., *Palmer 34787* (A); Stoddard Co., *J. A. Steyermark 5111* (MO); Taney Co., *Steyermark 5538* (MO). OKLAHOMA: LeFlore Co., *G. W. Stevens 2771* (MO).

In the original description Ashe cites the date and type locality as May 7, 1924 on Pine[y] Creek near Fort Douglas, Johnson County, Arkansas. A number of specimens of this collection annotated by Ashe are widely distributed in herbaria. The best of these syntypes is hereby designated as the lectotype. This type is No. 7 of Ashe's collections and is located at the New York Botanical Garden. The specimens collected by B. F. Bush near Eagle Rock, Missouri were also cited by Ashe in his original description as a second locality for this variety.

M. acuminata var. *cordata* (Michx.) Sarg., Amer. Jour. Sci. ser. 3, **32:** 743. 1886.

M. cordata Michaux, Fl. Bor-Amer. **1:** 328. 1803.

Tulipastrum cordatum (Michx.) Small, Fl. Southeast. U. S. 451, 1331, 1903.

M. acuminata var. *alabamensis* Ashe, Torreya **31:** 37. 1931.

Leaf thin and nearly membranous or thick and rugose; typically ovate-elliptic to oblong-obovate, with or without a short abrupt apiculus; apex rounded to acuminate or apiculate; base broadly acute-truncate to subcordate, rounded or cuneate; lower surface closely tomentose; petioles pubescent on adaxial surface, slender, 1.5–3 cm. long. Twig pubescent throughout and remaining so until spring of second year, or usually pubescent or roughened with bases of hairs during second season, red-brown turning gray in second or third year. The pubescence of the twig may be abundant and loosely appressed or scattered and tightly appressed. Flower often glaucous, greenish-yellow or with inner petals a clear golden yellow (Wax yellow; Ridgway, 1912) and outer petals less deep than inner (about Citron yellow); petals 2.5–7 cm. long and 1.5–3.5 cm. broad, obovate; peduncle 1.5–2.5 cm. long, typically villous with long white hairs or rarely glabrous.—Distributed over a relatively narrow range in the Piedmont and rarely Coastal Plain, from central North Carolina to central Alabama (Fig. 6, triangles).

ALABAMA: Baldwin Co., *Charles Mohr*, 2/6/87 (MO); Lee Co., *W. H. Duncan 9181* (GA); Pickens Co., *Duncan 15470 & J. W. Hardin* (GA); Shelby Co., *Mohr*, 9/27/1881 (A); Tuscaloosa Co., *S. J. Smith 4998 et al.* (GA). FLORIDA: Walton Co., *H. Kurz*, 9/3/48 (NCU). GEORGIA:

Baldwin Co., *E. T. Newsome*, 4/14/46 (GA); Bibb Co., *Berckmans*, 3/29/27 (A); Clarke Co., *Duncan 1420* (GA); Columbia Co., *Duncan 13554* (GA); Elbert Co., *Duncan 10555* (GA); Hancock Co., *Duncan 11027* (GA); Harris Co., *Duncan 6837* (GA); Hart Co., *Duncan 7791* (GA); Jones Co., *Berckmans*, 4/5/29 (NY); Lincoln Co., *Craig Orr E-4663* (GA); McDuffie Co., *Orr*, 7/30/36 (GA); Madison Co., *Duncan 11594* (GA); Morgan Co., *Duncan 7732* (GA); Muscogee Co., *Duncan 9660* (GA); Oconee Co., *J. H. Miller*, 4/10/32 (NCU); Oglethorpe Co., *Duncan 5535* (NCU); Putnam Co., *Duncan 7386* (GA); Richmond Co., Berckmans, 4/1913 (A); Stevens Co., *Duncan 10039* (GA); Stewart Co., *R. M. Harper 2227* (MO); Talbot Co., *Duncan 8479* (GA); Taliaferro Co., *Duncan 11368* (GA). NORTH CAROLINA: Anson Co., *W. B. Fox 2936 & S. Boyce* (NCU); Moore Co., *R. E. Wicker et al.*, 4/29/44 (NCU); Randolph Co., *Fox 1353 et al.* (NCU); Stanley Co., *Fox & R. K. Godfrey 1405* (GA); Union Co., *Boyce 1074* (GA). SOUTH CAROLINA: Aiken Co., *W. W. Ashe*, 8/1931 (NCU); Anderson Co., *L. B. Gibbs*, 7/1855 (NY); Laurens Co., *D. D. Jones*, 3/1944 (NCU); McCormick Co., *Duncan 10997* (GA); Newberry Co., *W. C. Coker*, 4/25/47 (NCU); Richland Co., *F. M. Monts*, 8/8/39 (NCU); Spartenburg Co., *Mrs. Phifer*, 4/21/35 (NCU).

The type specimen is preserved in the Museum National d'Histoire Naturelle of Paris (Sargent, 1894) and has not been seen by the author.

Coker (1943) presented a clear and interesting account of the long history of this variety. Much of the apparent confusion has been due in part to the descriptions in earlier manuals and their inclusion of forma *aurea* as previously mentioned. It is interesting to note that Sargent reduced *cordata* to a variety in 1886, but considered it a distinct species in his Manual of 1933. This change took place after the rediscovery of the natural populations by Berckmans in 1913. Rehder, likewise, treated this entity as a variety in 1927 and a species in his revised edition of 1940.

Even though the leaf was originally described as "foliis cordatis" and F. A. Michaux (1819) later illustrated a cordate-type leaf, the specific epithet was considered a misnomer by Coker (1943) who stated that this variety only rarely includes subcordate leaves. The statistical analysis, to be discussed later, substantiates this statement. Subcordate is more typically the shape of var. *ozarkensis*.

Ashe described var. *alabamensis* from specimens he collected in Choctaw and Tuscaloosa counties of Alabama in 1926. The type specimens have not been located by the author. Also mentioned in the original description were records from Geneva and Pickens counties, Alabama. Ashe first referred to these as *cordata*, but later separated them on the basis of the flower color (Coker, 1943). Ashe considered typical *cordata* as having the canary yellow flowers and *alabamensis* the greenish to yellow-green flowers.

The pubescence of the twigs in the Alabama collections is essentially identical with *cordata* collections in Georgia and South Carolina. The variety *alabamensis*, therefore, appears only as a western extension of *cordata*. It is interesting to note that Little (1953) includes *alabamensis* with var. *acuminata* rather than with *cordata*. Twig pubescence is apparently a more constant and dependable character than flower color, thus it seems that *alabamensis* is more closely related to *cordata*. The greenish flowers are by no means found only in the western populations of *cordata*, but may appear in any area where *acuminata* and *cordata* overlap in range. The Alabama populations thus represent a somewhat intermediate condition between *cordata* and *acuminata*.

TABLE 1

Individual comparisons, group comparisons and analysis of variance. ($\bar{x}$, mean; n, number; V, variance; s, standard error; t, test of significance; $V_{\bar{d}}$, variance of differences between means; $s_{\bar{d}}$, standard error of difference.)

		ozarkensis		cordata		acuminata	
		I	II	I	II	I	II
base	$\bar{x}$	4.	4.6	8.3	9.1	6.9	8.2
	n	24.	11.	19.	28.	131.	103.
	V	8.45	8.58	6.	1.24	5.75	9.85
	s	2.91	2.93	2.46	1.12	2.4	3.14
petiole	$\bar{x}$	3.	3.7	2.3	2.1	2.7	2.7
	n	24.	9.	15.	27.	131.	98.
	V	4.9	1.07	1.6	2.06	0.36	0.65
	s	2.22	1.04	1.27	1.44	0.6	0.8
pubesc.	$\bar{x}$	1.4		5.4		4.5	
	n	35.		46.		228.	
	V	8.75		0.79		1.67	
	s	2.96		0.28		1.29	
		l.	w.	l.	w.	l.	w.
petal	$\bar{x}$	4.2	1.2	5.	2.3	5.8	2.1
	n	7.	7.	20.	20.	80.	80.
	V	0.91	0.18	1.35	0.48	0.73	-
	s	0.3	0.13	1.16	0.69	0.86	-

		ozark.—cordata		ozark.—acuminata		cordata—acuminata	
		I	II	I	II	I	II
base	n	43.	39.	155.	131.	150.	114.
	$V_{\bar{d}}$	7.5	11.9	6.2	10.5	5.86	9.9
	$s_{\bar{d}}$	0.822	1.22	0.54	0.69	0.58	0.99
	t	5.2	3.7	5.37	1.3*	2.41*	3.63
petiole	n	39.	36.	155.	107.	146.	125.
	$V_{\bar{d}}$	0.38	0.44	0.34	0.7	0.29	0.56
	$s_{\bar{d}}$	0.2	0.25	0.128	0.091	0.15	0.165
	t	3.5	6.4	2.34*	11.	2.73	3.7
pubesc.	n	81.		263.		274.	
	$V_{\bar{d}}$	0.85		1.5		1.5	
	$s_{\bar{d}}$	0.2		0.22		0.16	
	t	20.		14.		5.6	

*1-5% level of significance. All others above 1%.

STATISTICAL ANALYSIS

From the data collected by methods previously described, various statistical calculations are used to show the variation within each variety and within each character, and to test the significance of the means and correlations.

Table 1 gives the results of these analyses. The mean, standard deviation,

and variance were found for four of the important characters for each variety. Leaves were analyzed by position I and II, and no significant difference was found between those from the sterile and fertile twigs; however, general observations showed greater variation among the leaves from different positions on the same twig.

The figures for base refer to the outlines (Fig. 1) and indicate a more or less characteristic shape for each variety, with *acuminata* as intermediate between *cordata* and *ozarkensis*. Also the means for the characters of petiole length and pubescence of lower leaf surface indicate *acuminata* as intermediate. The means for petal length and width show *acuminata* as the longest, *cordata* as intermediate and *ozarkensis* as the shortest and most narrow. These individual means are mere indicators of general tendencies. They are not at all useful for the purpose of identifying isolated specimens since the variabilities of each character overlap among the three varieties. The differences between means are represented by the polygonal graph (Fig. 3).

Group comparisons among the three varieties (lower half of Table 1) in all cases show a significant population difference. These differences were significant at the 1% level except those indicated by an asterisk which were significant to the 5% level. The Roman numerals used in the table indicate leaf position.

The pubescence of the peduncle is not included in the Table but does show a definite difference among the varieties. Of the peduncles examined, the following percentages were pubescent or villous: 0% of 15 in *ozarkensis*, 18.8% of 96 in *acuminata* and 79.4% of 34 in *cordata*. This character, although not originally used in making the determinations, seems to be quite characteristic and significant.

More significant than actual petal length or width is the trend in proportion shown by a collection from a number of flowers. The lines of regression (Fig. 4) show the trends in proportion for petal length vs. petal width in each variety. Anderson (1951) points out that closely related species of most plants differ in trend in proportion and that this is a particularly characteristic species difference. It is of interest here that these varieties should show trends which are characteristically different from one another. Perhaps trend in proportion is characteristic of any entity regardless of taxonomic level.

The test of significance for the regression line of *ozarkensis* (one with fewest specimens) was significant at a level indicating that the trend in each of the other two varieties is in itself significant. These lines show that the petals of *ozarkensis* are proportionately smaller and those of *cordata* are proportionately wider and more distinct than the other two. The heavy lines correspond to the actual range of variation in length among the individuals measured; the means are indicated by + on the lines. The wide variation in lengths may be due in part to differences in the degree of maturity at which the flower was collected. It seems too much to assume that all collections were of only mature flowers.

The geographical distribution of leaf pubescence between the tomentose variety of the east and the glabrous one of the Ozarks points up many interesting possibilities concerning the origin of the pattern of morphological variation. A

phenocontour map of pubescence (Fig. 5) has been drawn by using the mean pubescence within artificial populations or quadrats. These have been laid out over the entire range of the species and are 640 square miles each. The number of specimens examined is approximately 1 per 150 square miles, which should present an adequate picture of the distribution of pubescence over the entire area. Two isophenes were drawn, one between values 1 and 2, and the other between 4 and 5. The exact picture which these isophenes present is questionable in a few places since the mean for a quadrat was not always based upon a large number of specimens. A comparison with Fig. 6 indicates a relative lack of data from the Coastal Plain of Louisiana, Mississippi and Tennessee. For this reason, the southerly extension of mean 4 into Alabama and Mississippi has to be disregarded. The general pattern elsewhere is significant.

The general shape of the isophenes outlines the ancient Ozarkia and Appalachia—sparse pubescence in the Ozarks, an intermediate condition predominately northern and Appalachian, and dense tomentum in the Piedmont and Coastal Plain. The origin of this pattern may possibly be explained in many ways; however, it is conceivable that a species of once greater distribution persisted in the Ozarkian and Appalachian refugia during the great submergence period of late Cretaceous times. Upon the withdrawal of the Cretaceous seas during the early Tertiary, eastern populations of tomentose character migrated in all directions, although westward to the greatest extent, finally joining the glabrous form of the Ozarks and producing the intermediate pubescence type now distributed northward and in the Appalachians.

As previously shown, the var. *acuminata* is somewhat intermediate between *cordata* and *ozarkensis* in three morphological characteristics and geographically. A very simple interpretation may be that *acuminata* originated from the probable vicariads *ozarkensis* and *cordata* after the land between refugia became available for habitation. A thorough cytological study of the species, which may throw some light on this hypothesis, is long overdue. Dr. J. J. Westfall of the University of Georgia has made some cytological observations of *acuminata* and *cordata* (Correspondence, 1954) finding that they are indistinguishable as far as meiotic chromosomes are concerned. No record of a cytological investigation of *ozarkensis* has been found by the author. The species is known to be tetraploid (Whitaker, 1935); however, whether the varieties are allo- or auto-tetraploid is not known.

In summary, and to answer the questions stated in the Introduction, it may be said that the pattern of variation, as shown by a general morphological study treated statistically, indicates that *Magnolia acuminata* is composed of three segregates (considered here as varieties) and one color form. The origin of these segregates may be only hypothesized.

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NOTES ON SOME RARE NORTH CAROLINA PLANTS[1]

BY LIONEL MELVIN

Pleasant Garden, North Carolina

This paper is a record of some rarer plants that I have collected in this state. My identifications have been checked by Dr. H. R. Totten or by Dr. A. E. Radford of the University of North Carolina, and collections placed in the Herbarium of the University at Chapel Hill.

Lindera melissaefolium (Walt.) Blume (= *Benzoin melissaefolium* (Walt.) Nees). Bladen Co., near White Oak. July 2, 1939. The habitat is like that described by Thomas Walter, who discovered the plant. It is growing on the margin of a pond subject to drying up in seasons, but where the roots are at times submerged. There is no evidence that the plants are reseeding themselves, although they bear fruits. Reproduction is by stolons, a characteristic not common in the more abundant plant, *L. Benzoin*.

I believe that this Bladen County location is the only one known to exist in North Carolina. The New York Botanical Garden has, besides a duplicate of my 1939 collection, a very old collection received from the Princeton University Herbarium marked "Chapel Hill. Professor Mitchell"; and an old collection

[1] Presented before the Botany Section of the 51st Annual Meeting of the N. C. Academy of Science at Greenville, May 7–8, 1954.

from the Torrey Herbarium marked "North Carolina. Schweinitz." I have also examined from the U. S. National Herbarium the following C. L. Boynton collections from swamps near Hope Mills, Cumberland Co., N. C.: *Benzoin reticulatum* No. 967154 (Biltmore Herb. No. 14949[a]), No. 1435572 (Biltmore Herb. No. 14949[a]), No. 1435573 (Biltmore Herb. No. 8000), No. 1435574 (Biltmore Herb. No. 8000), and No. 1435575 (Biltmore Herb. No. 8903); these collections are not *Lindera melissaefolium*, but belong to the pubescent form or variety of *Lindera Benzoin* to which Palmer and Steyermark have given the varietal name of *pubescens*. Of this variety they say (Annals Mo. Bot. Gard. **22:** 545. 1935): "The occurrence of this undescribed pubescent variety has been the cause of considerable confusion and has been responsible for the wide range given in manuals to *Benzoin melissaefolium* (Walt.) Nees which has been credited to Missouri, but which appears to be a rare species confined to coastal plain and piedmont regions of the southeastern states." I find that the pubescent form or variety of *Lindera Benzoin* is not uncommon, and from the amount of winterkill of *L. melissaefolium* in the Bladen County plants in the coastal plain I doubt its existence farther north or even in the Piedmont of North Carolina; and I think that the notation "Chapel Hill" on the Mitchell plant meant only that it was sent by Professor Mitchell who was teaching at Chapel Hill over a hundred years ago.

Litsea aestivalis (L.) Fern.—July 2, 1939, in the same location as *Lindera melissaefolium*. This is the only North Carolina collection in the Herbarium of the University of N. C., though there are other collections from South Carolina and from Florida. In the New York Botanical Garden there are specimens from North Carolina as follows: Loomis, 1834; Wilmington (no date); Beyrich (113), Carolina, in nemoribus arenosis, 1836. Fernald (Rhodora **47:** 140–142, 1945) says: "So far as we know *Litsea aestivalis* has not been found in Virginia since Pursh collected it in Southampton County." Bladen County, N. C. is probably the most northern known location of *Litsea* at present.

Carya laciniosa (Michx.) Loud.—Guilford Co., summer and fall, 1953, along the swamp of South Buffalo Creek, near Greensboro. This hickory was reported from Beaver Swamp, Guilford Co., by E. H. Hall, September 10, 1931. No other locations east of the mountains are known except Cameron Park, Hillsboro, Orange Co., N. C., where H. R. Totten collected specimens from two trees in 1947. He thinks that these two trees may be of the original planting made before the Civil War.

Cornus alternifolia L. f.—I have made collections as follows: Alamance Co., at the junction of two streams about two miles northeast of Kimesville, Aug. 15, 1953; Randolph Co., at the foot of the north slope of Carraway Mountain, April 11, 1954; Guilford Co., along a stream in the eastern section of the county near the Alamance County line, May 2, 1954. The University of N. C. Herbarium has specimens of this species from Crabtree Recreational Park in Wake County, collected by others, from probably the most eastern known location of the species in the state.

Pyrola rotundifolia var. *americana* (Sweet) Fern.—Guilford Co., just east of

Jamestown, under pines on a north-facing slope, on land once in cultivation, March 7, 1954; same county, along Reedy Fork Creek, about two miles east of Summerfield, under hardwoods, on a north-facing slope, April 15, 1954; Rockingham Co., about 10 miles south of Madison, under hardwoods, on a north-facing slope, April 15, 1954. In the Piedmont of North Carolina I also know of two locations in Randolph County, on the Smithwin Farm, near Liberty; and the Herbarium of the University of North Carolina has specimens from Orange County (Howells Spring, Chapel Hill, J. N. Couch, June 26, 1920; Tenney's Meadow, Chapel Hill, W. C. Coker, 1939); and from Durham County (H. L. Blomquist and D. L. Correll, May 8, 1938).

ENVIRONMENTAL EFFECTS ON CONIDIAL VARIATION IN SOME FUNGI IMPERFECTI[1]

BY T. W. JOHNSON, JR.[2] AND J. E. HALPIN[3]

It is an established fact that illumination often alters the behavior of some fungi. In some species, visible light enhances sporulation; conversely in others, light induces deleterious effects. Of particular interest, however, are the effects of visible light on the morphology and production of conidia (Harter, 1939). Both effects confront the taxonomist who must necessarily consider the influence of environmental factors on the structures used in identification.

Snyder and Hansen (1941) have shown that illumination has a marked influence on the taxonomic characters in some species of *Fusarium*, and it is reasonable to conclude that representatives of related genera could exhibit similar responses. Among the cultural manipulations which the writers have employed on selected species of Fungi Imperfecti, that of light seems to have had the most effect. Various strains of certain species have exhibited increases in sporulation and variations in conidial morphology, or both, under illumination from incandescent and fluorescent light of various intensities.

Thoroughly cognizant of the fact that environmental conditions other than light might have been responsible for the responses observed, we were primarily concerned with the additional influences of such factors as pH of medium and age of culture. The present study was designed to investigate three problems: the influence of light on conidial morphology, the determination of the limits of artificial illumination for maximum conidial production, and the effect of other environmental agents on the influence evidenced by illumination. These additional factors are: age of culture; temperature of incubation; pH, nutrient concentration, and amount of medium; relative humidity; strain differences; method

[1] A portion of this investigation was carried out during tenure of the authors at the Chemical Corps Biological Laboratories, Camp Detrick, Frederick, Maryland.

[2] Department of Biology, University of Mississippi, University, Mississippi.

[3] Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.

of inoculation, and substratum. A portion of this investigation has been abstracted (Johnson and Halpin, 1952).

EXPERIMENTAL PROCEDURE

At least two strains representing separate collections or isolations of the following fungi were used: *Alternaria solani*, *Curvularia lunata*, *Helminthosporium sativum*, *H. teres*, *Piricularia oryzae*, *Fusarium moniliforme*, and *Cercospora sp.* Throughout the investigations three conditions of illumination were employed: continuous illumination with incandescent or fluorescent light of intensities of 50, 100, 200, 500, and 1000 foot candles; alternate light and dark periods, and darkness. While band spectrum limits were not considered in this investigation, the peak of relative energy with incandescent light was between wavelengths of 610 and 730 mμ, whereas that of fluorescent light was 460–550 mμ.

In all repeated trials of the various experiments, pure, single-spore cultures were used. Throughout the course of the study cultures were periodically reisolated as single-spore lines. Isolates were propagated on one of two stock media: malted barley extract agar,[4] or 2 per cent rice polish agar (Henry and Andersen, 1948).

Except for that portion of the investigation in which culture age was the variable, all isolates were five weeks old when subjected to illumination. Petri plates were seeded by injecting 1 ml. of a conidial suspension containing 1–2 million conidia (1:10 dilution) or 1 ml. of a minced mycelium suspension containing 3–5 million particles (1:100 dilution) onto the medium, and spreading the inoculum over the entire surface by gentle rotation of the plate. Aseptic handling of the suspensions for seeding was effected by harvesting conidia with sterile, distilled water containing a few glass beads to scrape the colony surface (Johnson and Tucker, 1954). Aliquots were drawn from the stock culture with sterilized hypodermic needles and syringes. Mycelium was minced in a stainless steel, modified Waring Blendor cup, and withdrawn aseptically.

A variety of substrata were employed. Buffered (pH 7.0) agar media used were potato dextrose, rice polish, malted barley extract, rice leaf extract, maltose-dextrin-glycerol, yeast extract, 2 per cent peptone, 3 per cent barley extract, and two synthetic media formulated by Leaver et al. (1947). In addition, a semi-synthetic medium formulated by Miss Betty Linthicum (personal communication) was used.[5] Agar in the synthetic and semi-synthetic media was treated so as to be free of accessory growth factors.

[4] 25 g. ground malted barley per l. distilled water, heated in water bath to 30–40 deg. C., and held for 15 min., then raised to 70 deg. C., and held for 30 min. Filtered while hot, reconstituted to a liter, and 2 per cent agar added.

[5] Dextrose, 2 g.; peptone, 10 g.; thiamin, 0.05 mg.; riboflavin, 0.05 mg.; nicotinic acid, 0.05 mg.; biotin, 0.01 mg.; agar, 20 g. Distilled water to volume of 900 ml., medium adjusted to desired pH, and Sorensen buffer solution for this pH added to make 1 liter. The peptone was charcoal-treated to remove traces of nicotinic acid, biotin, and calcium pantothenate; pyridine was used to remove thiamin.

Whole-grain substrata tested were a 1:1 mix of oat and sorghum seed, a 1:1 mix of corn and sorghum, and barley, corn, wheat, rice, and sorghum alone. These media were prepared in a manner similar to that described by Andersen et al. (1947) with the exception that 10 grams, dry weight, of seeds or grains was the maximum used. In the case of the whole-grain media, the seeds were placed in Petri plates in a single layer to effect a medium more nearly comparable in exposed surface area to that of the semisolid media. Conidia from these media were harvested with carbon tetrachloride.

Cabinets for illumination of cultures consisted of heavy black muslin on four sides and bottom, with the source of illumination centered on ring stands or Flexaframe above the cabinet. The intensity of illumination was controlled by varying the light source wattage, and the distance of the source from the substrate surface. In no instance was more than a 300 watt bulb with external porcelain reflector used in the series of tests with incandescent lights. Fluorescent fixtures consisting of banks of 15 or 40 watt "Slimline" tubes gave intensities up to 1000 ft. c. depending on the number of tubes and their distance from the substratum. Intensities were determined initially (and checked daily) with a Weston illumination meter. New bulbs or tubes were used after every 36 hours of continuous use.

To minimize the radiant heat from the light sources, all cabinets were equipped with a slow speed fan circulating the air directly over the culture dishes. Dissipation of the heat energy resulting from light striking the surface of the medium was not controlled. The intensity of radiance at the surface level of the medium at any one point in the cabinets was constant. There was, however, some variation in foot candles at various points under the light sources. Maximum variation was ± 6 ft. c.

The influence of illumination (and other factors) was determined in cultures at room temperature and at 26 deg. C. In the case of fluctuating room temperature, dry bulb readings were taken three times daily, and the average recorded as indicative of the temperature directly under the light source at the level of the culture dishes.

Cultures incubated in darkness were kept in a light-tight incubator at 26 deg. C., or at room temperature under two thicknesses of black muslin. Alternate light and dark incubation was secured by exposing plates to the various intensities for eight hours, covering them with black cloth for sixteen hours, and repeating this procedure for the seven day incubation period. Exposure of the cultures to north window light, without additional illumination or covering, was a second method of obtaining diurnal variation.

Harvesting conidia from semisolid media for counts and observations was accomplished by flooding the plates with 10 ml. of water, adding a few glass beads, and agitating the plates gently for two minutes. Throughout the investigation, eight replications were used for each treatment and environmental factor being tested. With one exception, each experiment was carried through ten replications. Four samples for conidial counts or morphological observations were taken from each culture dish. Incubation was of seven days duration, at

which time conidial counts were obtained by the use of the Howard mold counting cell.[6]

Additional cultural variations will be discussed in the sections reporting the results of the influence of those factors.

RESULTS

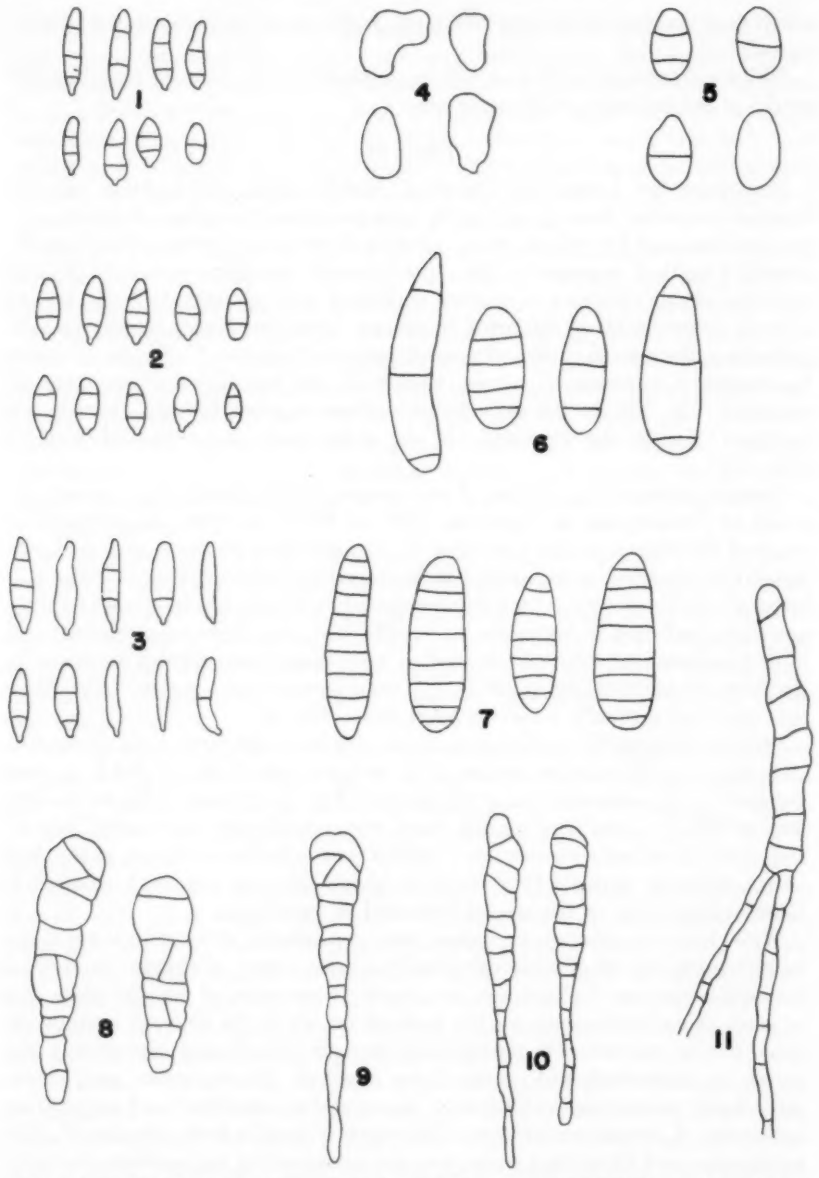
INFLUENCE OF LIGHT ON CONIDIAL MORPHOLOGY. Illumination effected changes in conidial shape in all strains tested of *Alternaria solani*, *Helminthosporium sativum*, and *Piricularia oryzae*. Conidia of the former species (three strains) showed a marked response to light. The "typical" muriform conidium (Fig. 8) was consistently obtained in cultures incubated in continuous darkness, and to a lesser extent in those subjected to diurnal variation. Under increasing light intensities, the conidia produced were elongate and narrow. Under the strongest fluorescent or incandescent source (1000 ft. c.) the conidia were invariably attenuated (Fig. 10). In the majority of cultures exposed to light there was a tendency toward the formation of an attenuated, once-branched rostrum (Fig. 11).

Conidia produced by cultures of two strains of *Helminthosporium sativum* exposed to incandescent or fluorescent light of 500 ft. c., were characteristic of those of the fungus on the host (Fig. 7). Conidia from cultures incubated continually in darkness were invariably shortened and aborted (Fig. 4). The majority of conidia produced by colonies exposed to 50 and 100 ft. c., and to alternate light and dark in intensities up to 500 ft. c. were short, single-celled or at most biseptate, but were not aborted as were those from cultures incubated in the dark. At an intensity of 200 ft. c. conidia approached the size of the "typical" ones, but generally possessed fewer septa (Fig. 6).

Striking changes in conidial shape were obtained with *Piricularia oryzae*. Conidia produced by cultures incubated in artificial light of 50 and 100 ft. c. were the most nearly characteristic of the species (Fig. 2). Colonies subjected to 200, 500, or 1000 ft. c. produced conidia which were considerably attenuated (Fig. 3), and were occasionally non-septate. Conidia from colonies incubated in the dark varied from the elongate type found in higher intensity culture incubation to those characteristic of the species produced on host tissue.

While these morphological changes were consistently induced in some of the isolates used, not all of these exhibited the same degree of change. In *Helminthosporium sativum*, for instance, the degree of variation of conidial shape was to some extent determined by the medium on which the cultures were propagated during exposure. On potato dextrose agar (dehydrated, and freshly prepared) the aforementioned changes were observed. On rice polish agar, on the other hand, no aborted conidia were observed, but shortened and aseptate conidia were of common occurrence. The shape of conidia from colonies of *Alternaria solani* and *Piricularia oryzae* was not influenced by the medium. No variations in conidial shape were induced in cultures of these three species if cultures

[6] See "The Howard mold count method as applied to tomato products." Research Division, American Can Company, pp. 17–25.



FIGS. 1–11. Conidia from cultures in darkness and under artificial light of various intensities; all ×785. FIGS. 1–3. *Piricularia oryzae*. 1. Darkness. 2. 50 and 100 ft. c. 3. 200 and 500 ft. c. FIGS. 4–7. *Helminthosporium sativum*. 4. Darkness. 5. 50 ft. c. 6. 200 ft. c. 7. 500 ft. c. FIGS. 8–11. *Alternaria solani*. 8. Darkness. 9. 200 ft. c. 10. 1000 ft. c. 11. 500 ft. c.

were incubated in darkness at temperatures ranging from 15–35 deg. C. (5 degree increments).

INFLUENCE OF LIGHT ON THE PRODUCTION OF CONIDIA. The influence of light on numbers of conidia is illustrated in figures 12–17. Data of a similar nature were obtained in each of thirty-five replications of the experiment, using all strains of these isolates, propagated on four media.[7] Up to a point, continuous exposure to light increased the number of conidia, whereas the lowest light intensity consistently suppressed conidial formation in some strains on certain media.

Cultures of all isolates propagated in darkness exhibited a more profuse vegetative growth than did those cultures in the light. Generally, *Curvularia lunata* and *Helminthosporium sativum* were induced to sporulate earlier under illumination than in the dark. In the case of the former, for instance, sporulation under light began 13–16 hours after seeding and exposing the plates, as compared with the onset of sporulation at about 41 hours after seeding, and subsequent incubation in the dark.

The number of conidia produced by isolates of *Fusarium moniliforme* and *Cercospora sp.* was not influenced by light. A non-sporulating strain of *Helminthosporium teres* did not sporulate under any light intensity. Variations such as humidity, and age of culture, similarly failed to induce a response in these three taxa.

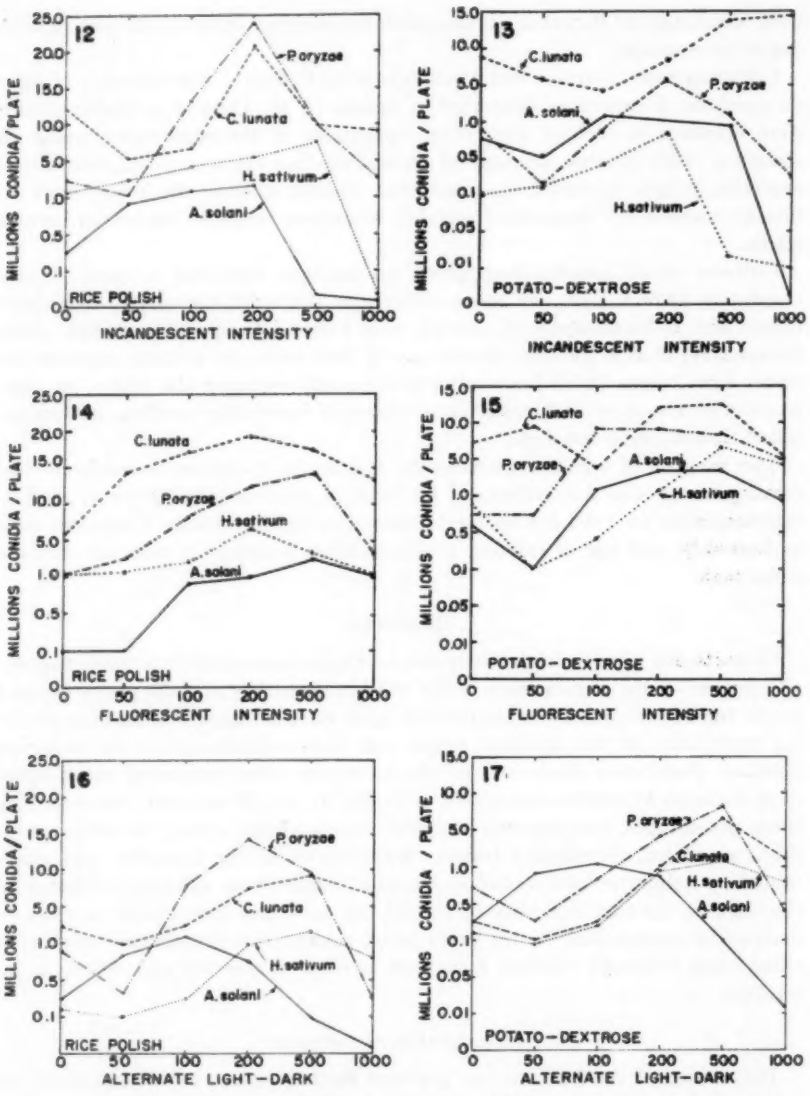
Humidity

Of particular concern in the early stages of experimentation was the deposition of moisture on the undersurface of the Petri plate covers of those plates exposed to the higher intensities of incandescent light. Such a deposition, and the resulting desiccation of the medium, might well have contributed to the observed increase. Tests were conducted in which cultures were incubated under lights or in darkness at relative humidities of 10, 25, 50, and 95 per cent. For obtaining these percentages, cultures were exposed, without plate covers, in surface-sterilized, air-cooled, illuminated transfer hoods in which the humidity was maintained by sulphuric acid solutions. Conidial counts from cultures incubated in the dark at the four humidity levels did not show any appreciable increase or decrease in sporulation. On the other hand, counts from illuminated colonies at these levels invariably showed a marked increase at the 200 and 500 ft. c. intensities.

Concentration of nutrients

Desiccation of the medium (see previous section) has an additional effect: an increase in concentration of the constituents. To determine whether or not this was responsible for the increases in sporulation under illumination, rice polish,

[7] While alternate light and dark incubation of cultures was made use of in all experiments, the counts are generally not reported. Conidial counts from these cultures were consistently intermediate between counts from colonies exposed to light, and those from colonies incubated in darkness.



FIGS. 12–17. Conidial production by colonies of *Alternaria solani*, *Helminthosporium sativum*, *Curvularia lunata*, and *Piricularia oryzae*, subjected to artificial illumination. FIGS. 12, 14, 16. On 2 per cent rice polish agar. FIGS. 13, 15, 17. On potato dextrose agar.

yeast extract, peptone, potato dextrose, and malted barley extract agars were prepared with total nutrient concentrations of ½, 1, 5, 10, and 20 per cent. In addition, the dextrose and peptone components in Miss Linthicum's medium were varied in these same percentages.

Counts from colonies on flood-seeded plates containing the various concentrations of medium were always higher when the fungi were illuminated, than were counts from those cultures not subjected to light. Although the concentration of nutrients did affect the total visible growth, sporulation was always increased by illumination, particularly at the 200 ft. c. level.

Substratum

Culture medium had the predominating influence on the amount of sporulation under illumination (Figs. 18, 19). The trend of results in other experiments in which light increased sporulation was again borne out by this phase of the experimental work.

Although all indications are that the influence of light is a quantitative one, results obtained with peptone and barley extract agars are suggestive of a qualitative effect. Henry and Andersen (1948) reported that on these media, *Piricularia oryzae* would not sporulate. Using subcultures of Henry and Andersen's strains, but exposing them to a continuous period of light, we obtained contradictory results (Table I). Sporulation, though meager, was induced by illumination, particularly at the 200 and 500 ft. c. level.

Amount of medium and pH

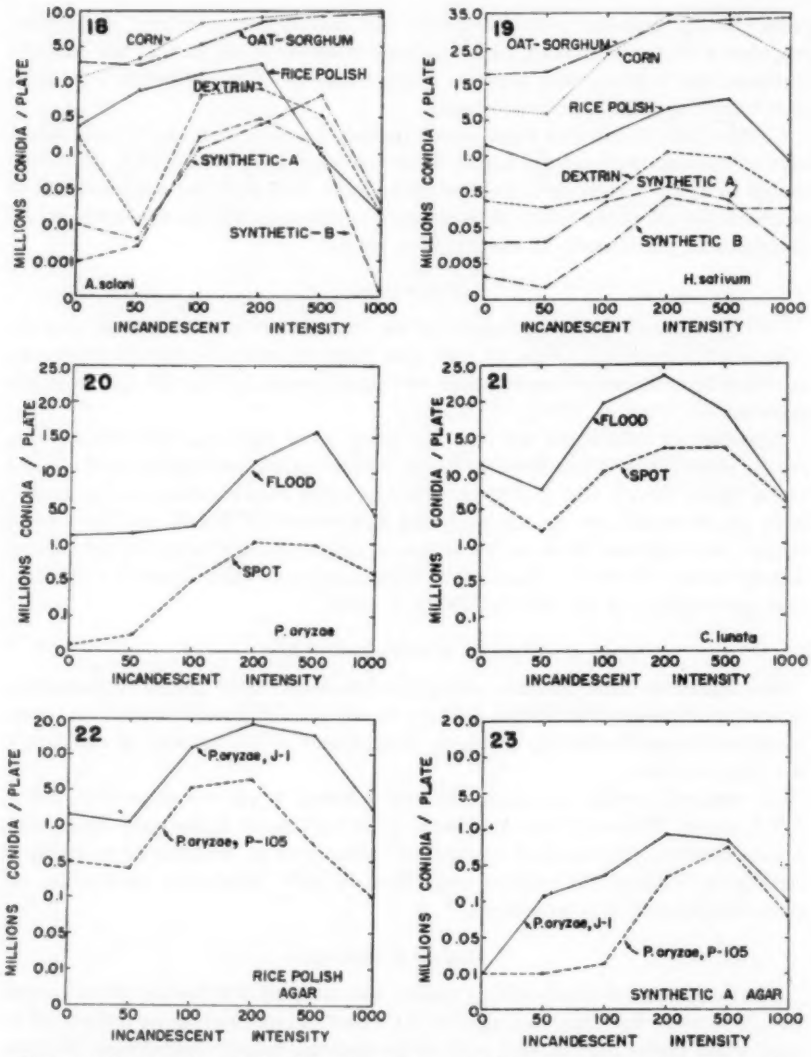
The influence of the amount (or depth) of medium on the degree of sporulation under light is negligible. Visible light up to 200 ft. c. increased sporulation (compared with non-illuminated cultures), irrespective of the amount of medium in the culture plates.

All semisolid media were adjusted and buffered to pH reactions of 5, 5.5, 6, 7, 7.5, and 8. While the isolates did not grow well in the higher acid range, with a consequent suppression of sporulation, there was an increase in numbers of conidia in illuminated cultures regardless of pH. Maximum production occurred at the 200 ft. c. intensity.

Method of seeding

The technique of flood-seeding plates was adopted for routine experimentation, but tests were run to compare the effect on sporulation as influenced by light when plates were seeded with single conidia, and by suspensions of these. The combined results of five series of tests with two species (Figs. 20, 21), are indicative of the influence of this variable.

There was a significant decrease in numbers of conidia from single spore colonies as compared with counts from colonies on flood-seeded plates. The influence of light was still in evidence, however, especially at the 200 ft. c. level. It appears, therefore, that colonies from single conidia are as markedly stimu-



FIGS. 18–23. Conidial production by colonies of *Alternaria solani*, *Helminthosporium sativum*, *Curvularia lunata*, and *Piricularia oryzae*, subjected to artificial light. FIGS. 18, 19. Influence of substrate on sporulation by *A. solani* and *H. sativum*. FIGS. 20, 21. Influence of method of seeding on sporulation by *P. oryzae* and *C. lunata*. FIGS. 22, 23. Strain reaction to illumination by *P. oryzae*.

TABLE I

Influence of illumination on the production of conidia by two strains of Piricularia oryzae *propagated on two media which do not support sporulation in cultures incubated in darkness or intermittent light.*

Counts in terms of thousands of conidia per plate.

Strain	Medium	Incandescent intensity (ft. c.)					
		0	50	100	200	500	1000
P-71	2% peptone	0	0	0.1	1.3	1.5	1.5
	3% barley extract	0	0	0	0.4	5.0	6.3
P-104	2% peptone	0	0	0.3	0.9	3.8	3.0
	3% barley extract	0	0.1	17.3	161.2	125.4	113.1

lated by light as are those developed in flooded plates. Again, the type of medium influenced the magnitude of increase under illumination.

Inoculation with "plugs" of mycelium and conidia from stock cultures, in comparison with flooded plates, exhibited a similar response. Plates inoculated with plugs developed colonies with fewer conidia produced than flood-seeded plates, but there was an increase in sporulation in colonies in both types of inoculated plates with exposure to artificial light.

Strain difference

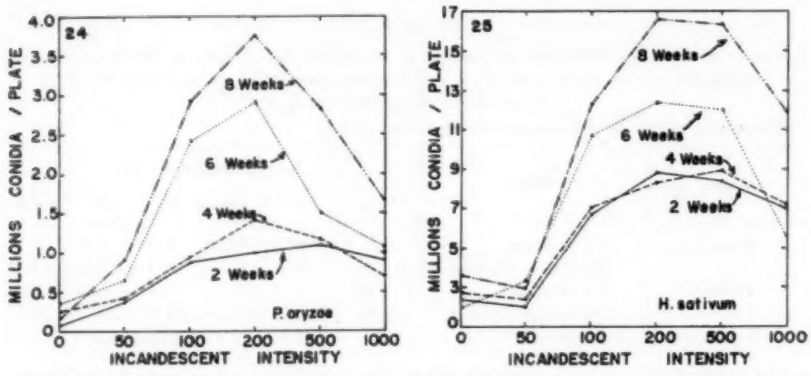
Strains of the isolates reacted differently (in amount of sporulation) when exposed to light. In *Piricularia oryzae*, for example, two strains differentiated on the basis of numbers of spores produced in stock cultures carried this difference over into cultures under illumination. A strain producing few conidia (P-105) retained this characteristic under illumination, but it could be induced to sporulate to a greater degree when colonies were illuminated. The more abundantly sporulating strain (J-1) exhibited a similar response (Figs. 22, 23). In cases where a spore ratio of 1 to 8 was characteristic of two strains in stock culture, this ratio was very nearly obtained when cultures were exposed to light, although the total number of conidia making up this ratio was increased.

Age of culture

The age of the colony affected the magnitude of sporulation under illumination. At an intensity of 200 ft. c., cultures six and eight weeks old invariably showed a considerable increase in numbers of conidia produced over those of cultures two and four weeks old (Figs. 24, 25). Older cultures of all strains of those species responding to light were more stimulated by light than were the younger ones.

Temperature

Preliminary tests indicate that the effect of temperature on illuminated colonies is one of an influence on the amount of growth, and hence on the ultimate numbers of conidia produced. At a constant incubation temperature of 26



FIGS. 24, 25. Conidial production, under artificial illumination, by cultures of *Piricularia oryzae* and *Helminthosporium sativum* at 2, 4, 6, and 8 weeks of age. FIG. 24. *P. oryzae*. FIG. 25. *H. sativum*.

deg. C., for example, all sporulating strains which had previously responded to light gave a two- to seven-fold increase in numbers of conidia at 100, 200, and 500 ft. c., over counts from plates incubated in darkness at the same temperature. Colonies subjected to incandescent or fluorescent light and incubated at 31 deg. C., gave spore counts which were generally only half as high as at 26 deg. C., but the increase in conidial production at 31 deg. C. was substantially the same.

Counts from illuminated cultures kept at room temperature were quite variable throughout the replications. In some instances the magnitude of increase at 200 or 500 ft. c. was five to twelve times that of cultures incubated in the dark at room temperature, while in other replications the increase was no more than four-fold. Incubation of isolates at a constant temperature under lights never resulted in such wide variation in conidial counts. Incubation of cultures without illumination, at temperatures ranging from 10 to 40 deg. C., resulted in conidial counts which were always lower than those from experiments with visible light.

By employing water-cooled light sources patterned after those described by Withrow et al. (1953), it was possible to increase incandescent illumination to 2100 ft. c. without increasing the air temperature above 25.5 deg. C. An increase in sporulation was in evidence up to 1500 ft. c., but the maximum production usually fell off beyond the 500 ft. c. level. Comparing this with the results obtained in cultures in the air-cooled cabinets at the same intensities, it is questionable that the variations in temperature in the air-cooled cabinets affected the results obtained.

At a constant temperature of 18 deg. C., sporulation was rather low, owing to slower growth of the organisms. In *Curvularia lunata* at this temperature, illumination did not increase conidial production, but in all other strains which previously showed a response to light, an increase was observed, with the peak of conidial production at 200 ft. c.

Quality of light

The results of this portion of the investigation showed little with respect to the influence of light quality on the amount of conidial increase. In some experiments and their replications fluorescent light was clearly more stimulative; in others, the reverse was true. The addition of band pass filter systems or long wave pass filters to the light sources is the next logical step toward resolving the light quality question.

DISCUSSION

The results reported confirm, in our opinion, the original supposition that light is stimulatory to the production of condidia in some strains of a limited number of dematiaceous Fungi Imperfecti. The variation of one or more cultural factors did not appreciably affect the increase in sporulation obtained with an increase in intensity of illumination. The temperature of incubation within the confines of the Petri plates under the light sources was the variable in the tests, and hence influenced the results but did not, it is felt, invalidate them.

It was observed in all strains but those of *Curvularia lunata* and *Fusarium moniliforme*, that colonies exposed to light consistently exhibited suppressed mycelial growth. This confirms the observations of Snyder and Hansen (1941), and raises the problem of how light effects the increase in numbers of conidia. It becomes a question of whether the suppression of mycelium results in a greater conidial yield (a phenomenon suggestive of increased sporulation with starvation), or whether the sporulation phenomenon itself is directly influenced photochemically. Speculating on the results obtained tends to support the latter premise. Strains of *Piricularia oryzae*, for example, which are characterized in ordinary laboratory stock culture by sparse mycelium and very high sporulation, show as marked increases in conidial production under illumination as do those strains characterized by luxuriant mycelium and either high- or low-count sporulation.

It seems advisable that cultures of some species (and perhaps all) of Dematiaceae be propagated in the presence of low intensity artificial light if maximum development of the colonies is to be realized. The taxonomist interested in genera of this group of fungi cannot perhaps adequately characterize or identify his fungi unless some cultures are propagated under illumination, and the morphological characteristics exhibited by such colonies taken into account.

SUMMARY

Seven species of dematiaceous Fungi Imperfecti were subjected to artificial incandescent or fluorescent light of 50–2100 ft. c. intensities. These were *Alternaria solani*, *Curvularia lunata*, *Helminthosporium sativum*, *H. teres*, *Piricularia oryzae*, *Fusarium moniliforme*, and *Cercospora sp.* Changes in conidial morphology of *A. solani*, *H. sativum*, and *P. oryzae* were induced by artificial illumination. An increase in numbers of conidia in all sporulating strains except those of *F. moniliforme*, *Cercospora sp.*, and in some instances in *C. lunata*, was concomitant with an increase of light intensity up to 1500 ft. c. The peak of conidial production occurred at the 200 or 500 ft. c. level.

While substratum had the most pronounced effect on the production of conidia in illuminated cultures, such environmental factors as pH, humidity, and nutrient concentration had little or no influence. Method of seeding and age of culture influenced the ultimate degree of sporulation under illumination. Temperature has a stabilizing effect on the amount of vegetative growth, hence on the numbers of conidia produced by illuminated colonies. That light has a quantitative rather than a qualitative effect on the strains tested, is indicated.

ACKNOWLEDGMENTS

The writers wish to express their sincere appreciation to Dr. A. J. Riker for his careful and critical editing and evaluation of the manuscript, and to Mrs. J. C. Rice for similar assistance. Evaluation of, and assistance in the preparation of the paper by Dr. J. E. Mitchell is greatly appreciated.

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JOURNAL

OF THE

Elisha Mitchell Scientific Society

VOLUME 70
1954

This Society was founded in 1883 through the efforts of
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Published for the Society
by the
University of North Carolina Press

CONTENTS OF VOLUME 70

Some Homoptera from the Caroline Islands. *Z. P. Metcalf* 1

The Status of the Names Sciurus niger cinereus Linnaeus and Sciurus niger vulpinus Gmelin. *Frederick S. Barkalow, Jr.* 19

Observations on the Abundance, Food, and Feeding Behavior of the Newt, Notophthalmus viridescens viridescens (Rafinesque), in Virginia. *John T. Wood and Ollie K. Goodwin* 27

On a Property Related to Separability in Metric Spaces. *F. Burton Jones* 30

A Note on Linear Spaces and Unicoherence. *Edward E. Grace* 33

Magnetic Observations on the South Carolina Coastal Plain. *H. W. Straley, III, and W. F. Straley* 35

A New Species of Myriotrichia Harv. from the Coast of North Carolina. *H. L. Blomquist* 37

Notes on Nearctic Hepaticae. VIII. Lejeuneaceae Holostipae of North America. *R. M. Schuster* 42

Sarracenia leucophylla Rafinesque. *C. Ritchie Bell* 57

The Characeae of Lake Mattamuskeet, North Carolina. *R. D. Wood* 60

The Genus Scirpus in North Carolina. *E. Dan Cappel* 75

A Synopsis of Jatropha, Subsection Eucurcus, with the Description of Two New Species from Mexico. *Robert L. Wilbur* 92

Range Extensions in the Flora of North Carolina—II. *Albert E. Radford* 101

Proceedings of the Fifty-First Annual Meeting of the North Carolina Academy of Science 105

Proceedings of the Elisha Mitchell Scientific Society 134

The Life History of the Northern Greenside Darter, Etheostoma blennioides blennioides Rafinesque. *William E. Fahy* 139

Structure and Relationships of the Anglerfish, Lophius americanus. *Theodore H. Eaton, Jr., Charles A. Edwards, Margaret Ann McIntosh, and Joseph Perry Rowland* 205

An Experimental Study of the "Barred" Pigment Pattern in Mexican Axolotl Larvae. *H. E. Lehman* 218

A Second Record of Myotis leibi in North Carolina. *Albert Schwartz* 222

Vitamin A and Tumor Growth in Gonadectomized, Methylcholanthrene-Injected Mice. *B. Lionel Truscott* 222

Comments on the Teaching of Trigonometry. *T. F. Hickerson* 228

The Utilization of Carbon and Nitrogen Compounds by Phytophthora parasitica Dastur var. nicotianae (Breda De Haan) Tucker. *Wirt H. Wills* 231

Sporangium Formation by Phytophthora parasitica Dastur var. nicotianae (Breda De Haan) Tucker. *Wirt H. Wills* 235

Toxicity as a Factor in Tobacco Black Shank. *Frederick T. Wolf and Frederick A. Wolf* 244

The Transpiration of Black Shank-Infected Tobacco. *Robert J. Schramm, Jr. and Frederick T. Wolf* 255

Respiration of Tobacco Black Shank Tissues. *Frederick T. Wolf and Robert J. Schramm, Jr.* 261

The Carbohydrate and Nitrogen Metabolism of Black Shank-Affected Tobacco. *Frederick A. Wolf and Frederick T. Wolf* 264

An Interpretation: Sexuality of Fungi. *Frederick A. Wolf* 269

The Biological Tenet "Alternation of Generations." *Frederick A. Wolf* 289

WHAT ARE FUNGI? *Frederick A. Wolf* 294
AN ANALYSIS OF VARIATION WITHIN MAGNOLIA ACUMINATA L. *James W. Hardin* 298
NOTES ON SOME RARE NORTH CAROLINA PLANTS. *Lionel Melvin* 312
ENVIRONMENTAL EFFECTS ON CONIDIAL VARIATION IN SOME FUNGI IMPERFECTI. *T. W. Johnson, Jr. and J. E. Halpin* 314

Vol. 69, no. 2, pp. 63–256, was issued on December 29, 1953.
Vol. 70, no. 1. pp. 1–104, was issued on June 18, 1954.

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RESPIRATION OF TOBACCO BLACK SHANK TISSUES
Frederick T. Wolf and Robert J. Schramm, Jr. 261
THE CARBOHYDRATE AND NITROGEN METABOLISM OF BLACK SHANK-AFFECTED TOBACCO ..... *Frederick A. Wolf and Frederick T. Wolf* 264
AN INTERPRETATION: SEXUALITY OF FUNGI ..... *Frederick A. Wolf* 269
THE BIOLOGICAL TENET "ALTERNATION OF GENERATIONS"
Frederick A. Wolf 289
WHAT ARE FUNGI? ..... *Frederick A. Wolf* 294
AN ANALYSIS OF VARIATION WITHIN MAGNOLIA ACUMINATA L.
James W. Hardin 298
NOTES ON SOME RARE NORTH CAROLINA PLANTS ..... *Lionel Melvin* 312
ENVIRONMENTAL EFFECTS ON CONIDIAL VARIATION IN SOME FUNGI IMPERFECTI ..... *T. W. Johnson, Jr. and J. E. Halpin* 314

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